ST. CLAIR RIVER ORGANICS STUDY

THE SCREENING OF INDUSTRIAL EFFLUENTS FOR GENOTOXIC ACTIVITY

MAY, 1981

Ministry of the Environment

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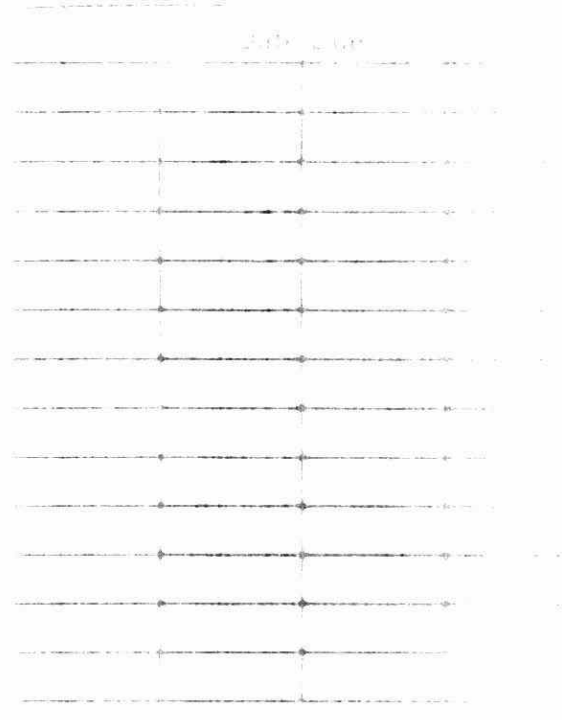

Ontario

Ministry
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Laboratory Services Branch
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MEMORANDUM: July 3rd, 1981.

TO: See Distribution List

FROM: Mr. Gerard C. Ronan
Director
Laboratory Services Branch

RE: MERCURY AND MUTAGEN REPORTS

Two studies on the St. Clair Lake System, The Status of Mercury Concentration of Fish from Lake St. Clair, and The Screening of Industrial Effluents for Genotoxic Activity, have been completed.

Since 1970, the Ministry of the Environment and the Ministry of Natural Resources, have carried out a joint program of monitoring fish for mercury levels in the St. Clair System. Over 10,000 fish have been analyzed, and the mercury report summarizes the information gathered over the past ten years.

Many of the fish species now have mercury concentrations low enough to meet federal guidelines for commerical fish sale, and the commercial fishery in Lake St. Clair has re-opened for many of the species. Ministry scientists predicted that a significant decline in mercury levels would occur as a result of the abatement activities undertaken by industry in the early seventies, and it is heartening to note that these projections have been borne out.

One of the objectives of the St. Clair River Organics Study was to determine, using bacterial tests, the mutagenic properties of organic compounds in various industrial and municipal effluents discharged to the St. Clair River. The report entitled "The Screening of Industrial Effluents for Genotoxic Activity" describes the results of laboratory testing of 25 industrial effluent sources, 5 intake waters and 5 river locations within the St. Clair River System.

The Screening of Industrial Effluents for Genotoxic Activity, is a follow-up of the Ontario Ministry of the Environment's 'St. Clair River Organic Study'. Six studies have been completed which include; The Detection of Mutagenic Activity: Screening of Twenty-three Compounds of Industrial Origin: Biological Surveys 1968 and 1977:

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Waste Dispersion: Identification and Quantitation of Organic Compounds: Fish Toxicity and Tainting Evaluations of Selected Industrial Effluents and Biodegradation of Organic Compounds.

For further information on the mercury report, contact Mr. B. Neary (248-3775) and for the mutagen report, contact Dr. D. Rokosh (248-3008).

Gerard C. Ronan

Mr. Gerard C. Ronan

GR/hc

EXPLANATION NOTE

The Screening of Industrial Effluents for Genotoxic Activity.

One of the objectives of the St. Clair River Organics Study is to determine, using bacterial tests, the mutagenic properties of organic compounds in various industrial and municipal effluents discharged to the St. Clair River.

Scientists at the Ontario Ministry of the Environment, have developed testing capabilities to achieve this objective. Two bacterial systems, the Ames Salmonella mutagenicity assay and the Rosenkranz DNA damage assay were used to detect genotoxic properties in effluent and water samples. These genotoxic properties damage or alter genetic information and result in an permanent heritable genetic change in bacterial cells. It has been shown through other research that substances genotoxic to bacterial systems are potentially mutagenic and/or carcinogenic in other biological systems, including man.

The report entitled "The Screening of Industrial Effluents for Genotoxic Activity" describes the results of laboratory testing of 25 industrial effluent sources, 5 intake waters and 5 river locations within the St. Clair River system. In the Ministry's Toronto laboratory, samples collected from these sites were processed by a chemical extraction technique which recovered and concentrated volatile organic compounds to levels considerably higher than those normally found in these industrial samples. None of the samples, in their unconcentrated state, gave a positive response to the mutagenicity or DNA damage assays. Some of the samples, however, when concentrated, yielded positive results. On the basis of tests conducted on sample concentrates, 18 of 25 effluent sources 5 intake waters and 5 river stations failed to induce a response in either the mutagenicity or DNA damage assays. Mutagenic activity was confirmed and DNA damaging activity was detected in samples concentrated from one effluent source from Dow Chemical of Canada Limited. Mutagenic activity was detected in concentrates of two additional effluents from this industry as well as in a concentrated sample of the final effluent of Ethyl Corporation of Canada Limited. Only DNA damaging activity was detected in concentrated effluent from each of Dow Chemical of Canada Limited, Polysar Limited and Imperial Oil Enterprises Limited.

This study was an extension of a previous work described in the report entitled "The Detection of Mutagenic Activity; Screening of Twenty-three Compounds of Industrial Origin", November 1979. This earlier work documented the detection of bacterial mutagenic activity in the chemically pure form of five compounds identified in effluents discharged to the St. Clair River-dichloromethane; 1,1-dichloroethane; 1,2-dichloroethane; 1,2-bromochloroethane; 1,2,3-trichloropropane. An additional compound 1,2-dichloropropane was identified in this more recent study as also mutagenic to the bacterial system. In two industial effluents, mutagenic activity in the concentrated effluent sample was related to the chemical

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identification of at least three compounds identified as mutagenic in their chemically pure form. Chemical mixtures are anticipated to induce a different response than such compounds in their chemically pure form. Thus, it is premature to conclude that the mutagenic activity detected in these effluents was due solely to the presence of these compounds.

This report concludes that while the detection of mutagenic activity in some effluent discharges was a matter of concern, there was insufficient evidence at this stage to predict that this activity was hazardous to man or the environment. It is emphasized that this activity was detected in concentrated effluent samples where levels of volatile organic compounds were considerably greater than present in the actual discharges. Moreover, the bacterial tests used in this study were capable of detecting genotoxicity but more extensive tests utilizing mammalian systems are necessary to assess the severity of any hazard. The report recommends that the results of this study be used in the setting of priorities for a more extensive evaluation of genotoxic hazards in industrial effluents.

The report recommends that additional studies be conducted to assess the hazard posed by the discharge of mutagenic substances into the environment. Specific research areas identifed included: the development of methods to test other chemicals, particularly polar organic compounds which may be present in such effluents; the development of a battery of tests permiting the verification of mutagenic hazards; the development of methods to assess the persistence, degradation and bioaccumulation of mutagenic substances discharged into the environment.

"The Screening of Industrial Effluents for Genotoxic Activity" is a follow-up of the Ontario Ministry of the Environment's 'St. Clair River Organics Study'. Six studies have been completed which include: "The Detection of Mutagenic Activity; Screening of Twenty-three Compounds of Industrial Origin", "Biological surveys 1968 and 1977", "Waste Dispersion", "Identification and Quantitation of Organic Compounds", "Fish Toxicity and Tainting Evaluation of Selected Industrial Effluents" and "Biodegradation of Organic Compounds".

ST. CLAIR RIVER ORGANICS STUDY

THE SCREENING OF INDUSTRIAL EFFLUENTS FOR GENOTOXIC ACTIVITY

MICROBIOLOGY SECTION
and
ORGANIC TRACE CONTAMINANTS SECTION
LABORATORY SERVICES BRANCH
ONTARIO MINISTRY OF THE ENVIRONMENT

MAY, 1981

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Summary

Effluents from industries along the St. Clair River downstream of Sarnia, Ontario, were tested for the presence of mutagenic and/or DNA damaging activity. These industries included: Imperial Oil Enterprises Limited, Polysar Limited, Dow Chemical of Canada Limited, Sunoco Incorporated, Shell Canada Limited, Ethyl Corporation of Canada Limited, Dupont Canada Incorporated, Canadian Industries Limited, and Petrosar Limited. Samples of influent water to three of these industries and samples of St. Clair River water from five locations in the vicinity of Polysar Limited and Dow Chemical of Canada Limited were tested for mutagenic activity.

A concentration procedure, which selectively recovered volatile organic compounds, was applied to all samples. The concentrated samples were tested for mutagenic activity using a modification of the Ames Salmonella mammalian microsome mutagenicity assay and for DNA damage in Eschericia coli using a modification of the Rosenkranz Pol A/Pol A^- DNA repair assay.

Neither mutagenic activity nor DNA damaging activity was detected in 18 of 25 effluents, or in the 5 influent waters tested.

Mutagenic activity was detected amd confirmed and DNA damaging activity was detected in samples taken from the Dow Chemical of Canada, 2nd Street Sewer effluent.

Mutagenic activity was detected and confirmed in the final effluent of the Ethyl Corporation of Canada Limited and 4th Street Sewer of Dow Chemical of Canada Limited. Mutagenic activity was detected in an initial sample but not confirmed in subsequent samples from the Dow Chemical of Canada Limited, DOEO effluent.

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DNA damaging activity was detected in the Stereo API effluent of Polysar Limited, the Pressure Sewer final effluent of Imperial Oil Enterprises Limited and the Acid Tile effluent of Dow Chemical of Canada Limited.

Concentrated samples from four St. Clair River locations failed to induce a mutagenic response. The response from a fifth sample, taken from the plume of the Dow Chemical of Canada Limited, 2nd Street Sewer, was inconclusive.

In an additional study, samples from three effluents of Dow Chemical of Canada Limited were analysed for the presence of volatile organic compounds using gas chromatgraphy/mass spectrometry. A total of 23 volatile organic compounds were identified in these three effluents. The list of identified compounds were then evaluated for their ability to induce a mutagenic response in the Ames test. Four of these volatile organic compounds, including methylene chloride, 1,1-dichlorethane, 1,2-dichloroethane and 1,2-dichloropropane, when tested in their chemically pure form in our laboratory, were found to contain mutagenic or presumptive mutagenic activity in the Salmonella test.

The detection of mutagenic and/or DNA damaging activity in some St. Clair River industrial effluents must be considered as a detection of a possible genotoxic hazard. The scope of the genotoxicity tests employed in this study does not permit the assessment of a risk from this activity to man or other species in the environment. Moreover, these tests were performed on a mixture of volatile organic compounds which was concentrated by

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a factor 350 to 500 fold greater than their levels in these effluents. Thus, the results of this report are best used in the setting of priorities for a more extensive investigation of the potential genotoxic hazard associated with industrial effluents.

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ACKNOWLEDGMENT

This report was prepared by D. A. Rokosh. The results presented here were principally obtained through work of D. A. Rokosh, R. D. Smillie and J. E. Pagel.

Appreciation is expressed to C. Tuzi and M. Pitcher for the concentration of the samples, to M. G. Foster for organic chemical analyses, to T. N. Lovasz and I. Smith for assistance in the genotoxicity testing, to J. Munro, T. Pawson and G. Szober for collection and delivery of samples, and to M. F. Salamone for suggestions and critical comments regarding this manuscript.

Sincere appreciation is expressed to G. C. Ronan, L. T. Vlassoff and O. Meresz for assistance in direction and constructive participation in planning and review of this study.

INTRODUCTION

A 1978 registry of the American Chemical Society identified more than four million chemicals of which forty-four thousand are believed to be in common use in North America. While many of these chemicals have undoubtedly improved the quality of life, some have also been shown to be hazardous(1). Considerable scientific work has been devoted to the identification of hazardous chemicals with recent efforts being applied to the detection of substances possessing toxic and genotoxic (mutagenic and/or carcinogenic) properties. The initial effort has concentrated on the detection of potentially hazardous substances. The ultimate goal is the characterization of risk factors associated with such substances and their restriction to levels which pose minimal risk to man and the environment.

The definite identification of a genotoxic hazard and assessment of its risk is best achieved through epidemiological studies on the human population. Such a general approach would be too complex and time-consuming to be practical. As an alternative, short-term genotoxicity tests have been developed. When applied as a battery of tests, incorporating screens for possible mechanisms of genetic damage, such tests can be used in the rapid identification of a potential genotoxic hazard. No single short-term test is completely effective in defining a substance as hazardous. A battery of short-term tests should provide sufficient evidence to verify a substance as a potential genotoxic hazard. The results of such tests are best used in defining the hazard and in the setting of priorities for a more extensive definition of risk.

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A common application of short-term genotoxicity tests has been the identification of potentially mutagenic hazards in commercial products. Such tests may also be applied to substances discharged as waste into the environment. Genotoxic compounds if present in these wastes could pose a hazard to exposed biological species. A concentration of these genotoxic substances in the environment would magnify the hazard which may ultimately affect even man, particularly if such hazardous substances accumulate in the food chain.

The St. Clair River Organic Study Group established a goal which was "to assess the presence and significance of organic compounds in the St. Clair River system and to establish from this program recommendations for control measures and further studies that may be required in relation to human health and environmental effects" (2, page i). One of the objectives of this study was to determine the potential genotoxicity of these organic compounds. A preliminary study had identified a number of organic compounds entering the St. Clair River. Twenty-three of these compounds, were tested and five in their chemicallly pure form were found to be mutagenic in a bacterial assay(2). A follow-up study was designed to determine if short-term tests could detect genotoxic activity in effluent sources which were expected to contain mixtures of organic compounds.

This report describes the results of genotoxicity testing of the concentrates of industrial effluents entering the St. Clair River. Genotoxicity tests for mutagenic and DNA damaging activity were conducted using a modified Ames and Rosenkranz test respectively. The concentration stage in the

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analysis permitted the selective testing of only the volatile organic compounds in the effluents. Samples eliciting positive activity in one or both of the genotoxicity tests were considered a detection of a potential genotoxic hazard.

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METHODS

Site Selection and Collection of Samples

Effluents, from industries along the St. Clair River downstream from Sarnia, Ontario, were studied. Those industries investigated had previously been selected by the St. Clair River Organic Study Group. The majority of effluents discharged by these industries into the St. Clair River were, where possible, tested for genotoxic activity.

Effluent samples were collected at the "end of pipe". One or four litre grab samples were taken in pre-cleaned one and four litre bottles with aluminum foil or Teflon lined caps. To minimize loss of the volatile component of the sample, bottles were completely filled to eliminate the head space, and were stored in the dark at 4^0C until concentrated. Samples were taken from the St. Clair River in areas influenced by effluent discharges of Dow Chemical of Canada and Polysar Limited. Grab samples of four litre volumes were collected from the river at one meter depths. Treatment and storage of river samples were the same as for the effluent samples.

Concentration of Volatile Organics

Samples collected from the effluents and the river were concentrated by the "Purge and Trap" method(3). Generally one litre samples of the effluents and four litre samples from the St. Clair River were concentrated.

The concentration method was based on the principle of purging the volatile organics from solution with an inert gas and trapping these compounds on an adsorbent. The organic compounds were eluted from the adsorbent with dimethylsulphoxide (DMSO). Eluates from the traps were collected in glass vials.

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The vial head space was eliminated by adding pre-cleaned glass beads and the vials were sealed with a Teflon lined screw cap. These concentrates were stored, for up to four weeks, in the dark at 4^0C. Eluate volumes, in the range of 2.0 to 3.0 mL were recorded and are presented in the results. The efficiency of this concentration method, evaluated on a standard mixture of nine volatile organic compounds, was described in a previous report(3).

Test for Mutagenic Activity

Samples were tested for mutagenic activity using a modification of the Ames Salmonella typhimurium mutagenicity test(4). Because of a limitation in concentrated sample volume, testing was restricted to tester strains TA 98 and TA 100.

Bacteriological Tester Strains

The tester strains were obtained from Dr. Bruce Ames, Berkeley, California. Master cultures of TA 98 and TA 100 were maintained frozen at -60^0C in Nutrient-saline broth plus 8%(v/v) DMSO. Subcultures (holding cultures) were maintained for up to one month at 4^0C on Nutrient-saline agar in plates sealed to prevent dehydration. Twenty-four hour broth cultures of the tester strains were used in the assay. Broth cultures were prepared in Nutrient-saline broth supplemented with 0.8 mg Ampicillin/30mL. This broth was inoculated with several colonies from the holding culture and incubated at 37^0C for 24 hours.

Quality Control of Tester Strain

The cultures were checked before each test for purity by a streak on Nutrient-saline agar, and for Ampicillin resistance,

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ultraviolet sensitivity and crystal violet sensitivity by the method recommended by Ames et al (4). Biochemical requirements were examined at monthly intervals. When any one of these quality control checks were not met, the culture was rejected and fresh cultures were isolated from the frozen master culture. In addition, all plates in the test were examined for contaminating colonies, whose presence also resulted in rejection of the test. The spontaneous or background mutation frequency for each tester strain was determined on solvent control plates.

Preparation and Delivery of Mutagenic Standards

Positive controls (known mutagenic compounds) were incorporated into the test to assess the response of the tester strains to a known genotoxic agent. The compound N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) was used for strain TA 100 with and without S-9. The compound 2-aminofluorene (2AF), was used to assess the response of strain TA 98 in the presence of S-9.

The mutagenic compound was weighed in a sealed vial, using a differential weighing technique. The compound was dissolved in a volume of DMSO to give a final concentration of 1.0 mg/mL. The solution was diluted further to give the desired concentration in 10 µL of DMSO solution. Positive control solutions were initially stored for 2 to 3 weeks in the dark at 4^0C. The storage time was reduced to one week in later tests.

Positive standard solutions were delivered to the positive control tubes in 10 µL aliquots using a 1000 µL micropipetter with disposable tips.

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Mutagenicity Testing of Effluent Concentrates

Effluent concentrates were tested for mutagenic activity using the pour plate technique(4). This technique employed a top agar solution of 100 mL volume which was supplemented with 10 mL of 0.5 mM l-histidine and 10 mL of 0.5 mM biotin. This mixture was dispensed in 2.0 mL aliquots into test tubes held at 42^{0}C in a dry-block heater. The contents of these test tubes were supplemented by the addition of, in order: a) 100 mL tester strain broth culture, b) DMSO, positive control or effluent concentrate, and c) when required, 0.2 mL S-9 reaction mixture. The contents in the tubes were thoroughly mixed, then poured on the surface of Vogel-Bonner agar plates(4). Each sample was tested on strains TA 98 and TA 100 with and without S-9. An analysis for each sample consisted of a) positive control containing 2AF for TA 98 plus S-9, b) a positive control containing MNNG for TA 100 with and without S-9, c) a solvent control containing DMSO at a volume equal to the largest volume of concentrate tested to a maximum of 250 µL, and d) three or four doses of effluent concentrate in a volume ranging from 25 to 250 µL. Poured plates were divided into groups of solvent control, positive control and test plates containing identical volumes of effluent concentrate. Groups of plates were immediately transferred to stainless steel, petri-plate holders and these containers were sealed. All plates were incubated in the dark, at 37^{0}C for 48 hours.

The response of the tester strains was measured after the 48 hr incubation period by counting histidine positive revertant colonies which developed on the plates. The development of the bacterial "lawn" was also examined for toxicity.

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Metabolic activation of compounds in the effluent concentrate was attempted using rat liver S-9 enzyme. Two sources of S-9 product were used in the study. The first source was prepared from Aroclor 1254 induced, male Wistar rats by the method recommended by Ames et al(4). This lot of S-9 was employed on effluent samples taking during 1979. A second source of Aroclor 1254 induced S-9, obtained from Inter Medico, Willowdale, Ontario, was employed with samples taken in 1980. Rat liver S-9 was stored frozed at -60^{0}C, and once thawed, was held on ice and used within 4 hours.

The S-9 reaction mixture employed in the test contained per 1000 µL, a) sodium phosphate buffer (100 µmoles), pH 7.4, b) magnesium chloride(8 µ moles), c) potassium chloride(33 µ moles), d) nicotinamideadeninedinucleotide phosphate (4 µ moles), e) glucose-6-phosphate (5 µ moles) and f) Arcolor 1254 induced rat liver S-9(100 µL). Aliquots of 200 µL S-9 reaction mixture were used per plate.

A daily run consisted of the analysis of three samples. All effluent concentrates were coded by sample number by the Organic Trace Contaminants Section and the effluent source was not known until completion of the analysis.

Interpretation of Mutagenicity Test Results

Revertant colonies counts for each strain on the three solvent control plates were used to calculate an average solvent control number (C) for each run. Accumulated solvent control numbers for each strain, originating from the same holding culture, were used to calculate the average historical background revertant level (Cav.). The response induced by the sample in each tester strain was corrected for the background revertant level by subtracting C from the number of revertants counted on the test plates(E). Revertant colonies on test plates in excess

and in deficit of background were calculated. The mutagenic activity ratio (MUTAR) for each test plate was calculated by dividing the corrected revertant count (E-C) in excess of the solvent control by Cav.

The response induced by the samples were compared to the variability in revertant numbers in the solvent controls. A standard deviation and variance was calculated from these counts for each strain within each test run. The historical within-run variance for each strain was calculated from the accumulated solvent control numbers. Variability limits of the solvent control counts for each strain were set at the 95% confidence limit (n=3) of the historical within-run standard deviation (calculated from the historical within-run variance). A response was assumed different from the solvent control if revertant counts exceeded the variability limits of the solvent control.

Aliquots of concentrates which resulted in the clearing of greater than 75 percent of the bacterial lawn were assumed toxic to the strain and were scored "T". Samples resulting in a dose-related decrease in revertants below solvent control counts were also assumed toxic and were scored "(T)". Samples eliciting a dose-related increase in revertants exceeding the variability in the solvent control were suspected mutagenic and were scored "(-)". Samples eliciting a dose-related increase in revertants and a MUTAR value equal to or exceeding 1.5 were scored "(+)". Both of these responses were assumed to be mutagenic if a repeat analysis of the effluent produced a similar response. Samples demonstrating a dose-related increase in revertants and resulting in a MUTAR value in excess of 2.5 were scored "+" and

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were assumed to contain bacterial mutagenic activity.

Test for Bacterial DNA Damage

Effluent sample concentrates were tested for bacterial DNA damaging activity using a modified Rosenkranz test(5). The test relies on the principle that agents which result in DNA damage are potentially toxic to the bacterial cell. It is assumed that processes in the normal cell have the ability to repair the damage to DNA. DNA damaging activity is suspected if a compound is preferentially more toxic to a cell lacking a DNA repair mechanism.

The tester strains Escherichia coli W3110, which contains DNA repair mechanisms, and E.coli P3478, which is a mutant lacking the polymerase A DNA repair process, were obtained from Dr. H. Rosenkranz. Bacterial cultures were maintained at 4^0C as surface colonies on HA+T agar plates(5). Twenty-four hour broth cultures were used in the test. Such cultures were prepared by inoculating test tubes containing 3 mL HA+T broth with several colonies from the plate culture and incubating the tubes at 37^0C in a shaking water bath for 24 hours.

Effluent concentrates were tested for DNA damaging activity using tester strains W3110 and P3478 with and without S-9 and employing a spot plate technique. In the test, HA+T top agar, containing HA+T broth supplemented with 1.0% agar was dispensed in 2.0 mL aliquots into test tubes equilibrated to 42^0C in dry block heater. The tubes were supplemented with 100 µL of the 24 hour broth culture of the tester strain. Tests incorporating metabolic activation were supplemented with 200 µL S-9 reaction mixture, as described earlier in the text. The inoculated tubes were mixed thoroughly and the contents poured onto HA+T agar plates.

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Solvent control, negative control, positive control and test plates were run for each strain combination. A sterile, 13 mm Schleicher-Schnell filter paper disk was placed on the surface of the overlay for each solvent control and test plate. A sterile 7 mm disk was placed on the surface of the positive control plates. DMSO (100 µL) was added to the disks on the solvent control plates. A Dispenso-o-disk (Difco) containing 30 µg chloramphenicol was placed on the surface of the negative control plates. MNNG was added to the disks on the positive control plates. Disks on the test plates received 100 µL of the effluent concentrate.

Positive control and test plates were sealed in individual stainless steel petri dish holders while all other plates were grouped and sealed in polyethylene vegetable crispers. The containers were incubated in air at 37^0C for 24 hours.

After the incubation period, bacterial growth produced a bacterial lawn on the surface of the plate. Toxicity was indicated by the clearing (inhibition) of the lawn around the disk. The degree of toxicity was estimated by measuring the diameter of this zone of clearing. The zone of inhibition was calculated by subtracting the diameter (mm) of the disk from the diameter (mm) of the zone of lawn clearing around the disk.

The quality of the test was assured by the development of a uniform bacterial lawn, the absence of toxicity in the solvent control, similar toxicity on the negative control plate and an increase in toxicity in strain P3478 over that for strain W3110 on the positive control (MNNG) plate.

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Effluent concentrates failing to clear the bacterial lawn were considered non-toxic. Effluent samples resulting in an equal degree of clearing of the lawns of strains W3110 and P3478 were assumed to be toxic to these strains but to contain no DNA damaging activity. Samples resulting in an increased zone of inhibition in strain P3478 were assumed to contain bacterial DNA damaging activity.

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Results

A previous study had detected mutagenic activity in certain volatile organic compounds at doses ranging from 1 to 40 mg per plate (2). These mutagenic compounds had been identified in the St. Clair River system. However, it was assumed that levels of organic compounds in the St. Clair River industrial effluents would, at practical test volumes, be below the detection limits of our genotoxicity tests. Consequently, a method for the concentration of volatile organic compounds from industrial effluents, which afforded a 350 to 500 fold concnetration of these compounds, was developed by the Organic Trace Contaminants Section.

Compatibility of the Concentration Method with the Mutagenicity Test

The compatibility of this concentration method with the Ames test was evaluated on control samples consisting of DMSO eluted through the Tenax GC trap. The results of a bacterial mutagenicity test on these control samples is shown in Table 1.

These control samples were not toxic to the Salmonella tester strains. Bacterial toxicity, as indicated by a clearing of the bacterial lawn, was not observed at all doses tested to a maximum of 250 µL per plate. The control samples also failed to induce a dose-related increase in revertants in the Salmonella tester strains with and without S-9. This observation suggests the absense of detectable mutagenic activity in the elution solvent (DMSO) or in its eluates from the clean absorbtion resin. It was assumed therefore, that if mutagenic activity was detected in the effluent samples concentrated by this method, this activity would be due to organic compounds recovered from the effluent sample.

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Table 1. Mutagenic activity in control samples.

Sample Number		Revertants per plate											
		TA 98			TA 98 + S9			TA 100			TA 100 + S9		
27/6-3	C*	25	16	22	19	32	33	136	115	124	121	121	92
	MNNG[a] 2ug							6480	3900	2310	3180	5500	9360
	2AF[b] 2ug	15	26	22	338	300	525						
	250[c] uL		14			26			119			111	
	125		21			35			116			102	
	75		25			26			118			136	
	25		30			63			149			117	
24/6-4	C	16	6	5	47	31	40	247	241	309	166	170	174
	MNNG 2ug							435	480	1245	1260	4170	1620
	2AF 2ug	34	24	8	465	345	360						
	250 uL		10			32			266			144	
	125		10			34			190			170	
	75		16			28			199			189	
	25		20			53			194			180	
3/6-4	C	8	9	9	23	26	14	120	115	137	138	125	146
	MNNG 2ug							1410	3060	3000	2865	5565	5400
	2AF 2ug	21	9	17	1275	1320	1185						
	250 uL		18			35			132			147	
	125		11			26			102			123	
	75		9			50			118			133	
	25		19			22			159			157	

Table 1. continued.

Sample Number		Revertants per plate											
		TA 98			TA 98 + S9			TA 100			TA 100 + S9		
17/3-1	C	0	4	11	11	3	8	140	125	150	187	179	191
	MNNG 4ug							1037	533	1475	3690	1790	4575
	2AF 4ug	3	0	2	2257	1852	823						
	200 uL		4			8			166			178	
	100		0			9			183			179	
	50		11			13			177			211	
22/8-3,4	C	7	3	8	24	7	5	272	204	204	207	158	232
	MNNG 10ug							6300	7300	3000	>10000 **		
	2AF 2.5ug	16	21	26	3200	3600	3400						
	250 uL		18			26			65			98	
	100		9			20			159			190	
	50		11			22			98			137	
21/7-3	C	18	22	23	34	32	38	178	100	105	81	87	75
	MNNG 2ug							362	480	510	310	510	615
	2AF 2ug	43	55	45	1945	1659	1185						
	250 uL		24			26			84			109	
	125		41			36			84			143	
	75		38			35			102			107	
	25		21			83			80			88	

* Solvent control, DMSO at volume equal to the maximum aliquot of sample tested,
a N-methyl-N'-nitro-N-nitrosoguanidine, b 2-aminofluorene, c aliquot of sample tested,
** revertant count on all three positive control plates.

The response induced in tester strain TA 100 and TA 100 plus S-9 by these control samples indicated compatibility of the concentration method with this strain. Revertant colonies on plates, containing doses of the control sample ranging from 25 to 250 µL, were generally similar to revertant colonies counted on the respective solvent control plates. In one control sample (22/8-3,4), however, suppression of the revertant colonies below the solvent control level was observed. This suppression of revertant numbers was not dose-related and fell within variability limits for TA 100 solvent control during this stage of the study.

In TA 98, revertant colonies on plates containing these doses of the control samples were generally greater than those in the respective solvent control. However, TA 98 revertant numbers on these plates were within the variability limits of revertant numbers on the solvent control plates.

In TA 98 plus S-9, revertant numbers on plates containing these doses of the control samples were also greater than those on the respective solvent control. On plates containing 25 µL of two control samples (27/6-3 and 21/7-3), revertant numbers exceeded those of the solvent control by 35 and 48 colonies respectively. However, the responses induced by larger doses of these samples were within the variability limits of revertant numbers on the solvent control plates. Revertant counts on the plate containing the 75 µL dose of sample 3/6 -4 exceeded counts on the solvent control by 29 colonies but revertant counts on plates containing three additional doses of this sample were also similar to those of the solvent control. The increase

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in revertant numbers observed at individual doses of these three samples was not dose-related and thus was not assumed to be a sign of mutagenic activity. These results, however, suggest an additional variability in the response of strain TA 98 plus S-9. This variability was considered in the interpretation of test results obtained with concentrated effluent samples.

A consideration of this variability in the response of tester strain TA 98 plus S-9 was in part necessitated by low levels of revertant colonies on the solvent control plates. Historical average revertant numbers for the solvent control were 10 and 22 for TA 98 and TA 98 plus S-9 respectively and lower than the spontaneous revertant levels for this strain reported by Ames et al(4). However, quality control checks including ampicillin resistance and detection of mutagenic activity in 2AF, which were run during each test, confirmed the strain as TA 98.

In the design of the study, it was hoped that the mutagenic response induced by the concentrated effluent samples could be compared to revertant numbers on positive control plates. However, a wide within-run and between-run variability was observed in the response of the tester strain to the positive control compounds MNNG and 2AF. This variability may in part be explained by the response of biological systems to these mutagenic compounds. However, this variability may also be influenced by the technique used in the preparation and delivery of the mutagenic compounds to the positive control plates. In the preparation of these standards, milligram quantities of the mutagenic compound were weighed and solutions of these standards were delivered in 10 µL aliquots using a 1000 µL pipetter. Both practices would provide variability in the final concentration of these standards

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Table 2. Escherichia coli DNA damaging activity in control samples.

Sample	Zone of Inhibition* W3110 -S9	W3110 +S9	P3478 -S9	P3478 +S9
C**	0	0	0	0
MNNG[a] 5ug	3	2	12	14
CAMP[b] 30ug	19	18	16	16
21/1-1 (100 uL)	0	0	0	0
C	0	0	0	0
MNNG 10ug	7	6	18	18
CAMP 30ug	20	19	16	16
22/8-3,4 (100 uL)	0	0	0	0

* Diameter of the zone of inhibition in excess of disc (mm)
** Solvent control, DMSO 100 uL per plate
a N-methyl-N'-nitro-N-nitrosoguanidine
b Chloramphenicol

on positive control plates. Both 2AF and MNNG have steep dose-response curves(6) inducing a large increase in response with a small increase in dose. Because of this variability, the response to the positive standard was used to verify the ability of the tester strain to detect a known mutagenic compound, but the positive standard was not used as a reference for the response induced by the test sample.

Compatibility of the Concentration Method to the DNA Damage Test

Control samples, containing DMSO eluted through the Tenax GC trap, were subjected to a test for bacterial DNA damage. The results of this test is shown in Table 2. Aliquots of 100 µL of these control samples were non-toxic to E.coli strains W3110 and P3478. These results indicated the absence of detectable bacterial toxicity and bacterial DNA damaging activity in these control samples.

Genotoxicity Testing of Industrial Effluents

A listing of industries and their effluents tested for genotoxic activity is given in Table 3. This table also includes the sampling data, the concentrate number, the volume of sample concentrated and the final volume of the concentrated sample. The results of tests on these samples is presented on an industry by industry basis. None of the samples, in their unconcentrated state, gave a positive response to the mutagenicity or DNA damage assays. Some of the samples, however, when concentrated, yielded positive results.

Imperial Oil Enterprises Limited (IOEL)

Concentrated effluent samples from the #3 Separator, the Pressure sewer the #9 Separator and the Bio-oxidation System were tested for mutagenic activity. The results of these tests, are given in the Appendices IA through ID and these results are summarised in Table 4. The results of a test of these effluents

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Table 3. Documentation of concentrated samples of industrial effluent and influent sources tested for mutagenic and DNA damaging activity.

Industry	Concentrate Number	Date Sampled (DDMMYY)*	Sample Volume (L)	Volume of Concentrate (mL)
Imperial Oil Enterprises Limited				
Effluent				
#3 Separator	21/7-2	030780	1	2.4
	29/8-3,4	120879	1	2.0
Pressure Sewer	4/7-1	030780	1	2.4
	22/7-1		1	2.4
	29/8-5,6	210880	1	2.0
#9 Separator	4/7-2	030780	1	2.4
	22/7-2		1	2.4
	30/8-1,2 °	210879	1	2.0
Bio-oxidation System	21/7-1	030780	1	2.4
	29/8-7,8	210879	1	2.0
Influent				
Service Water	4/7-3	030780	1	2.4
	22/7-3		1	2.4

° DNA damage test only.

* Date expressed in DD(day), MM(month) and YY(year).

Table 3. continued.

Industry	Concentrate Number	Date Sampled (DDMMYY)	Sample Volume (L)	Volume of Concentrate (mL)
Polysar Limited				
Effluent				
Township Ditch Effluent	23/6-2	190680	1	2.4
	9/5-2	290480	1	2.2
	21/1-3 ° *	240879	1	2.8
54" Sewer	23/6-1	190680	1	2.4
	10/3-2	050380	1	2.2
	12/3-1		3	2.2
	18/1-2 ° *	160879	1	2.8
	23/8-1,2 °		1	2.0
66" Sewer	24/6-2	190680	1	2.4
	31/8-3,4 °	240879	1	2.0
Stereo API	25/6-1	190680	1	2.4
	27/6-2		1	2.4
	13/3-1	050380	1	2.2
	17/3-2		3	2.2
	22/8-1,2	160879	1	2.0
72" Sewer	24/6-3	190680	1	2.4
	27/6-1		1	2.4
	31/8-5,6 °	240879	1	2.0
Influent				
Township Ditch Influent	24/6-1	190680	1	2.4
	21/1-2 ° *	240879	1	2.8
Service Water	23/6-3	190680	1	2.4

° DNA damage test only.
* Sample stored five months before concentration.

Table 3. continued.

Industry	Concentrate Number	Date Sampled (DDMMYY)	Sample Volume (L)	Volume of Concentrate (mL)
Dow Chemical of Canada Limited				
Effluent				
42" Sewer	29/5-3	220580	1	2.4
	28/8-7,8°	230879	1	2.0
48" Sewer	30/5-1	220580	1	2.4
	27/8-5,6°	230879	1	2.0
Acid Tile	30/5-2	220580	1	2.4
	27/3-2	040380	1	3.0
	24/8-1,2°	150879	1	2.0
54" Sluice	30/5-3	220580	1	2.4
	29/8-1,2	230879	1	2.0
2nd Street Sewer	25/8-3	200880	1	2.4
	27/5-2	220580	1	2.4
	2/6-2		1	2.4
	23/6-4		1	2.4
	18/3-1	040380	1	2.2
	27/8-1,2°	230879	1	2.0
3rd Street Sewer	25/8-1	200880	1	2.4
	26/5-1	220580	1	2.3
	28.8-11,12°	230879	1	2.0
DOEO	27/5-1	220580	1	2.4
	28/5-2		1	2.4
	13/3-2	040380	1	2.2
	14/3-1		3	2.2
	28/8-9,10°	230879	1	2.0
4th Street Sewer	25/8-2	200880	1	2.4
	2/6-1	220580	1	2.4
	26/5-2		1	2.4
	27/8-3,4 °	230879	1	2.0
Steam Plant	3/6-2	220580	1	2.4
	29/5-2		1	2.4

° DNA damage test only.

Table 3. continued.

Industry	Concentrate Number		Date Sampled (DDMMYY)	Sample Volume (L)	Volume of Concentrate (mL)
Dow Chemical of Canada Limited					
Influent					
4th Street	2/6-3		220580	1	2.4
Service Water	27/5-3			1	2.4
3rd Street	3/6-3		220580	1	2.4
	29/5-1			1	2.4
Sunoco Incorporated					
Final Effluent	6/5-2		290480	1	2.2
	9/1-1	° *	210879	1	2.0
	21/1-2	*		1	2.8
Shell Canada Limited					
Final Effluent	8/5-2		290480	1	2.2
Contaminated Water	13/5-1		290480	1	2.2
Ethyl Corporation of Canada Limited					
Final Effluent	12/5-1		290480	1	2.2
	26/3-1		050380	1	3.0
	15/1-1	*	210879	1	2.8
	29/1-5	° *		1	2.8
Dupont Canada Incorporated					
Final Effluent	7/5-2		290480	1	2.2
	10/1-1	° *	230879	1	2.0
	24/1-3	° *		1	2.8
Canadian Industries Limited					
Final Effluent	7/5-3		290480	1	2.2
	21/1-3	° *	230879	1	2.8
Petrosar Limited					
Final Effluent	5/5-2		290480	1	2.2

° DNA damage test only.
* Sample stored five months before concentration.

for DNA damaging activity are given in Appendix X and are summarized in Table 5. The results of a mutagenicity test on the IOEL Service Water influent are given in Appendix IE and these results are summarized in Table 4.

Concentrates 21/7-2 and 29/8-3,4 from two samples of the #3 Separator effluent when tested at doses ranging from 25 to 250 µL, failed to elicit an increase in histidine positive revertants in tester strains TA 98 and TA 100 with and without S-9.(Appendix IA). An additional concentrate sample (28/9-3,4) when tested at 100 µL, was not toxic to Escherichia coli tester strains W3410 and P3478 with and without S-9.(Appendix X) Because of the absence of toxicity to strain P3478, this concentrate was scored negative for DNA damaging activity (Table 5).

Concentrates 21/7-1 and 29/8-7,8 from the Bio-oxidation System effluent also failed to induce a mutagenic response in tester strains TA 98 and TA 100 with and without S-9.(Appendix ID). DNA damaging activity was not detected in a 100 µL dose of concentrate 29/8-7,8 (Appendix X).

Duplicate concentrates 4/7-2 and 22/7-2 from a sample from the #9 Separator effluent failed to elicit a dose-related increase in revertants in strain TA 100 with and without S-9 (Appendix IC). The elevated revertant numbers detected at the 25 µL dose of concentrate 4/7-2 were within the variability limits of revertant number in the respective solvent control.

Concentrate 22/7-2 induced revertant counts in TA 98 which exceeded those on the solvent control by a factor of

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1.0 and 1.8 respectively. This response was not repeated when duplicate concentrate 4/7-2 was tested at similar doses. The response induced by concentrate 22/7-2 in TA 98 was considered to be due to variability in the test and not an indication of mutagenic activity.

In TA 98 plus S-9, the response induced by the 75 µL dose of concentrate 4/7-2 exceeded the solvent control by a factor of 0.6. Moreover, the response of the 250 µL dose of 22/7-2 in this strain exceeded the solvent control by a factor of 0.96. However, since the response to these duplicate concentrates was not repeated, it was assummed that the sample was not mutagenic in TA 98 plus S-9.

A test on concentrate 30/8-1,2 from a second sample of the #9 Separator effluent also failed to detect bacterial DNA damaging activity in E. coli (Appendix X).

Duplicate concentrates 4/7-1 and 22/7-1 of a sample as well as concentrate 29/8-5,6 of a second sample of the Pressure Sewer final effluent failed to elicit dose-related increase in revertant numbers in strain TA 98 with and without S-9 (Appendix IB). The elevated revertants induced at the 25 µL dose of 4/7-1 and the 125 µL dose of 22/7-1 were within the variability of revertant numbers on their respective solvent control plates. These concentrates also failed to induce a mutagenic response in TA 100 with and without S-9. However, a dose-related suppression in revertant numbers below those of the solvent control was induced by concentrate 29/8-5,6 in strain TA 100 and TA 100 plus S-9. Toxicity, as indicated by lawn clearing, was also observed in strains TA 98 and TA 100 at doses of 200 µL and 100 µL

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Table 4. Summary of the results of the Salmonella mutagenicity test on concentrated samples from Imperial Oil Enterprises Limited.

Effluent	Concentrate Number	Mutagenicity Score TA 98 -S9	TA 98 +S9	TA 100 -S9	TA 100 +S9
#3 Separator	21/7-2	-	-	-	-
	29/8-3,4	-	-	-	-
Pressure Sewer	4/7-1	-	-	-	-
Final	22/7-1	-	-	-	-
	29/8-5,6	T	T	T	T
#9 Separator	4/7-2	-	-	-	-
	22/7-2	-	-	-	-
Bio-oxidation	21/7-1	-	-	-	-
System	29/8-7,8	-	-	-	-
Influent					
Service Water	4/7-3	-	-	-	-
	22/7-3	-	-	-	-

Table 5. Summary of the results of the E. coli test for DNA damaging activity on concentrated samples from Imperial Oil Enterprises Limited.

Effluent	Concentrate Number	Toxicity	DNA Damage
#3 Separator	29/8-3,4	-	-
Pressure Sewer Final	29/8-5,6	+	+
#9 Separator	30/8-1,2	-	-
Bio-oxidation System	29/8-7,8	-	-

of this concentrate while clearing of the bacterial lawn at a dose of 200 µL was observed in TA 98 plus S-9 and TA 100 plus S-9.

Concentrate 29/8-5,6 from the Pressure Sewer final effluent was toxic at a 100 µL dose to E. coli strain W3110 and P3478. This toxicity was greater in strain P3478 than in strain W3110 indicating DNA damaging activity in this effluent sample.

Concentrated samples from the Service Water influent sample (4/7-3 and 22/7-3) failed to induce a mutagenic response in strain TA 100 with and without S-9 (Appendix IE). On strain TA 98, concentrate 22/7-3 at doses of 25, 75 and 125 µL induced revertant counts exceeding those of the solvent control by a factor of 2.0, 2.7 and 1.6 respectively. This response in TA 98 was not repeated at similar doses of the duplicate concentrate (4/7-3) from this sample. In TA 98 plus S-9, broth concentrates 22/7-3 and 4/7-3 failed to induce a response exceeding the variability of revertant numbers in the solvent control. Because the response in TA 98 could not be repeated in the duplicate concentrate, there was insufficient evidence to conclude mutagenic activity in this sample.

Polysar Limited

Concentrated effluent samples from the Township Ditch, the 54" Sewer, the 66" Sewer, the Stereo API and the 72" Sewer were tested for mutagenic activity. The results of the mutagenicity tests on these samples are given in the Appendices IIA through IIE and these results are summarized in Table 6.

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The results of tests for DNA damage on concentrated samples from these effluents are given in the Appendix XI and these results are summarized in Table 7.

The results of the mutagenicity test on concentrates of samples from the Township Ditch influent and the Service Water influent are given in the Appendices IIF and IIG respectively, and these results are summarized in Table 6. The results of the test for DNA damage on the Township Ditch influent are given in Appendix XI and these results are summarized in Table 7.

Concentrated samples from the Township Ditch effluent (Appendix IIA), the 54" Sewer effluent (Appendix IIB) and the 72" Sewer effluent (AppendixIIE) failed to elicit a mutagenic response in the tester strains TA 98 and TA 100 with and without S-9.

Concentrate 21/1-3 from the Township Ditch effluent, concentrates 18/1-2 and 23/8-1-2, from the 54" Sewer and concentrate 31/8-5,6 from the 72" Sewer, when tested at a dose of 100 µL, failed to induce DNA damage in E.coli(Appendix XI).

Concentrate 24/6-2 from a sample of the 66" Sewer effluent failed to induce a mutagenic response in strain TA 100 with and without S-9 as well as in strain TA 98(Appendix IIC). In strain TA 98 plus S-9, concentrate 24/6-2 at doses of 25 µL induced a response which exceeded revertant numbers solvent control by a factor of 1.9. The revertant numbers elicited by the 75 µL dose of this concentrate did not exceed the variability in revertant numbers in the respective solvent control. This response in TA 98 plus S-9 to this concentrate was considered to be within the variability of the test and not a mutagenic response.

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Table 6. Summary of the results of the Salmonella mutagenicity test on concentrated samples from Polysar Limited

Effluent	Concentrate Number	Mutagenicity Score TA 98 -S9	TA 98 +S9	TA 100 -S9	TA 100 +S9
Township Ditch Effluent	23/6-2	-	-	-	-
	9/5-2	-	-	-	-
54" Sewer	23/6-1	-	-	-	-
	10/3-1	-	-	-	-
	12/3-1	-	-	-	-
66" Sewer	24/6-2	-	-	-	-
Stereo API	27/6-2	-	-	-	-
	25/6-1	-	-	-	-
	13/3-1	-	-	-	-
	17/3-2	-	-	-	-
	22/8-1,2	-	-	T	(T)
72" Sewer	27/6-1	-	-	-	-
	24/6-3	-	-	-	-
Influent					
Township Ditch Influent	24/6-1	-	-	-	-
Service Water	23/6-3	-	-	-	-

Table 7. Summary of the results of the *E. coli* test for DNA damaging activity on concentrated samples from Polysar Limited.

Effluent	Concentrate Number	Toxicity	DNA Damage
Township Ditch Effluent	21/1-3	-	-
54" Sewer	18/1-2	-	-
	23/8-1,2	-	-
66" Sewer	31/8-3,4	-	-
Stereo API	22/8-1,2	+	+
72" Sewer	31/8-5,6	-	-
Influent			
Township Ditch Influent	21/1-2	-	-

A test on a 100 µL dose of concentrate 31/8-3,4 from a second sample of the 66" Sewer effluent failed to detect DNA damage in E.coli (Appendix XI).

Concentrated samples from the Stereo API effluent failed to induce a mutagenic response in tester strain TA 98 with and without S-9 (Appendix IID). Concentrates 27/6-2, 25/6-1, 13/3-1 and 17/3-2 from this effluent also failed to induce a mutagenic response in TA 100 with and without S-9. An additional concentrate 22/8-1,2 from this effluent induced a dose-related suppression in revertant counts in TA 100 and in TA 100 plus S-9 and was toxic to TA 100 at a dose of 200 µL. At a dose of 100 µL, this concentrate was also toxic in E.coli (Appendix XI). Moreover, increased toxicity to strain P3478 over W3110 indicated DNA damaging activity in concentrate 22/8-1,2 from the Stereo API effluent.

A concentrated sample of the Township Ditch influent (Appendix IIF) and a concentrated sample from the Service Water influent (Appendix IIG) failed to elicit a mutagenic response in strains TA 98 and TA 100 plus S-9. DNA damage was also not detected in a concentrated sample from the Township Ditch influent (Appendix XI).

Dow Chemical of Canada Limited

Concentrated samples from the Dow Chemical of Canada Limited 42" Sewer, 48" Sewer, Acid Tile effluent, 54" Sluice, 2nd Street Sewer, 3rd Street Sewer, Direct Oxidation of Ethylene Oxide (DOEO) effluent, and 4th Street Sewer were tested for mutagenic activity and DNA damaging activity. The results of

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the mutagenicity testing of these effluents are given in the Appendices IIIA through IIIH and are summarized in Table 8. The results of tests for DNA damage on these effluents are given in Appendix XII and are summarized in Table 9. The results of mutagenicity tests on samples of the Steam Plant effluent are given in the Appendix IIIK while the results of the mutagenicity tests on samples of the 4th Street Service Water and 3rd Street Service Water are given in the Appendices III I and IIIJ respectively. The summary of the results of the mutagenicity test on the later three samples are given in Table 8.

Concentrated samples from the 42" Sewer (Appendix IIIA). the 48" Sewer (Appendix IIIB) and 54" Sluice (Appendix IIID) failed to induce a mutagenic response in the Salmonella tester strains with and without S-9. Moreover tests on concentrated samples from 42" Sewer, 48" Sewer and the 54" Sluice failed to detect DNA damaging activity (Appendix XII).

A test in Salmonella on concentrates 30/5-2 and 27/3-2 from the Acid Tile effluent also failed to detect mutagenic activity (Appendix IIIC). The response induced in TA 98 and TA 98 plus S-9 by concentrate 30/5-2 and the response induced by concentrate 27/3-2 in TA 100 and TA 100 plus S-9 were within the variability of revertant number in their respective solvent controls. Concentrate 24/8-5,6 from an additional sample of the Acid Tile effluent was not toxic in E.coli strain W3110 but was toxic at 100 µL to strain P3478 (Appendix XII). Therefore, DNA damaging activity was concluded for this concentrated effluent sample.

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Table 8. Summary of the results of the Salmonella mutagenicity test on concentrated samples from Dow Chemical of Canada Limited.

Effluent	Concentrate Number	Mutagenicity Score TA 98 -S9	TA 98 +S9	TA 100 -S9	TA 100 +S9
42" Sewer	29/5-3	-	-	-	-
48" Sewer	30/5-1	-	-	-	-
Acid Tile	30/5-2	-	-	-	-
	27/3-2	-	-	-	-
54" Sluice	30/5-3	-	-	-	-
	29/8-1,2	-	-	(T)	(T)
2nd Street Sewer	25/8-3	-	-	-	-
	23/6-4	-	-	-	-
	2/6-2	-	-	(-)	(-)
	27/5-2	-	-	(-)	(-)
	18/3-1	-	-	(-)	-
3rd Street Sewer	25/8-1	-	-	-	-
	26/5-1	-	-	(T)	(T)
	28/8-11,12	-	-	(T)	(T)
DOEO	28/5-2	-	-	-	-
	27/5-1	-	-	-	-
	14/3-1	-	-	-	-
	13/3-2	-	-	-	-
	28/8-9,10	-	-	(- T)	(T)
4th Street Sewer	25/8-2	-	-	-	-
	2/6-1	-	-	(+)	(+)
	26/5-2	-	-	(-)	(-)
	27/8-3,4	-	-	(T)	(T)
Steam Plant	3/6-2	-	-	-	-
	29/5-2	-	-	-	-
Influent					
4th Street Service Water	2/6-3	-	-	-	-
	27/5-3	-	-	-	-
3rd Street Service Water	3/6-3	-	-	-	-
	29/5-1	-	-	-	-

Table 9. Summary of the results of the E. coli test for DNA damaging activity on concentrated samples from Dow Chemical of Canada Limited.

Effluent	Concentrate Number	Toxicity	DNA Damage
42" Sewer	28/8-7,8	-	-
48" Sewer	27/8-5,6	-	-
Acid Tile	24/8-1,2	-	+
54" Sluice	29/8-1,2	-	-
2nd Street Sewer	27/8-1,2	+	+
3rd Street Sewer	28/8-11,12	-	-
DOEO	28/8-9,10	-	-
4th Street Sewer	27/8-3,4	+	-

All concentrated samples from the 2nd Street Sewer failed to elicit a mutagenic response in strain TA 98 with and without S-9 (Appendix IIIE). However, duplicate concentrates 27/5-2 and 2/6-2 from a sample of this effluent induced a dose-related increase in revertant numbers in strain TA 100 (Appendix IIIE). Revertant numbers at the 250 µL dose of concentrates 27/5-2 and 2/6-2 exceed those in the solvent control by factors of 0.5 and 0.7 respectively. The ratio of revertant numbers in excess of background to the historical spontaneous revertant frequency (MUTAR) was 0.6 for the 250 µL dose of concentrate 27/5-2. The MUTAR value for the 250 µL dose of concentrate 2/6-2 was 0.5. A third concentrate (23/6-4) prepared from the same sample as 27/5-2 and 2/6-2, but concentrated four weeks later than 27/5-2 failed to induce a mutagenic response in TA 100. This result suggested that the substances responsible for the mutagenic activity was lost during storage of the sample. In TA 100 plus S-9, concentrates 27/5-2 and 2/6-2 also induced a dose-related increase in revertant numbers, which, at the 250 µL dose exceeded those of the solvent control by a factor of 1.35 and 0.67 respectively. MUTAR values at the 250 µL dose of 27/5-2 and 2/6-2 in this strain were 1.46 and 0.58 respectively. Concentrates 25/8-3 and 23/6-4 from the 2nd Street Sewer failed to induce a response in TA 100 plus S-9 in excess of the variability in their respective solvent control.

A concentrate (18/3-1) from an additional sample of 2nd Street Sewer elicited dose-related increase in revertant numbers in TA 100 but not in TA 100 plus S-9. In TA 100 revertant numbers at the 200 µL dose of 18/3-1 exceeded those of the solvent control by a factor of 0.8, and a MUTAR value of 0.75 was calculated for the 200 µL dose of this concentrate.

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Concentrate 27/8-1,2 from a sample of the 2nd Street Sewer was toxic to E. coli strain W3110 (Appendix XIII). Increase toxicity to strain P3478 over that to strain W3110 indicated DNA damaging activity in this effluent sample.

Concentrated samples from the 3rd Street Sewer,(25/8-1, 26/5-1 and 28/8-11,12) failed to elicit a mutagenic response in strain TA 98 (Appendix IIIF). In strain TA 98 plus S-9, concentrate 25/8-1 at a dose of 125 µL induced revertant numbers which exceeded those of the solvent control by a factor at 0.93. Concentrate 28/8-11,12 at doses of 50 and 100 µL induced TA 98 revertants over those of the solvent control by a factor of 0.95 and 1.05 respectively. Although, an increase in revertants of TA 98 plus S-9 was observed in two samples, this response was not considered to be of sufficient magnitude to indicate a mutagenic response.

In strains TA 100 with and without S-9, concentrate 25/8-1 from the 3rd Street Sewer failed to elicit a response differing from the variability in revertant numbers in the solvent control. However, concentrates 26/5-1 and 28/8-11,12 from this Sewer induced a dose-related suppression in TA 100 revertant numbers. This toxicity to Salmonella in concentates 28/8-11,12 from the 3rd Street Sewer was not observed in E. coli tester strains W3110 and P3478. DNA damaging activity was not detected in this sample (Appendix XII).

Concentrated samples from the Direct Oxidation of Ethylene Oxide (DOEO) effluent failed to elicit a mutagenic response in strain TA 98 (Appendix IIIG). The elevated revertant numbers induced in TA 98 plus S-9 by concentrates 28/5-2, 27/5-1 and 28/8-9,10 from this effluent were within the

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variability of their respective solvent controls and were also assumed not to indicate mutagenic activity.

Concentrate 28/8-9,10 from DOEO effluent at a dose of 50 µL induced a response in TA 100 which exceeded revertant numbers in the solvent control by a factor of 0.94 (Appendix IIIG). The MUTAR value at the 50 µL dose of this concentrate was 0.90. At a dose of 100 µL of this concentrate revertant numbers in TA 100 exceeded the solvent control by a factor of 0.68. Suppression in revertant numbers at the 100 and 200 µL dose of this concentrate was observed in TA 100 and TA 100 plus S-9. It was assumed that the response induced by concentrate 28/8-9,10 was a mutagenic response. However, concentrates 28/5-2, 27/5-1, 14/3-1 and 13/3-2 from the DOEO effluents failed to induce a mutagenic response in TA 100 with and without S-9. The mutagenic response observed in the initial concentrated sample (28/8-9,10) could not be confirmed in subsequent samples taken from the DOEO effluent.

A test for DNA damage on 100 µL dose of concentrate 28/8-9,10 failed to detect toxicity or DNA damaging activity in the E. coli (Appendix XIII).

All concentrated samples from the 4th Street Sewer effluent failed to induce a mutagenic response in strain TA 98 with and without S-9 (Appendix IIIH). However, in strain TA 100, concentrates 2/6-1 from this Sewer induced a dose-related increase in revertant numbers. At the 250 µL dose, this sample induced revertant number increases in TA 100 and TA 100 plus S-9 which exceeded those of their corresponding solvent controls by a factor of 2.15 and 1.58 respectively. The MUTAR value at this

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dose of 2/6-1 in TA 100 was 1.53 while the MUTAR value in TA 100 plus S-9 at this dose was 1.58. A duplicate concentrate (26/5-2) from this sample also elicited a dose-related increase in revertants in this strain. At the 250 µl dose of this concentrate, revertants of TA 100 and TA 100 plus S-9 exceed those of the solvent control by a factor of 0.86 and 0.84 respectively. MUTAR values in TA 100 and TA 100 plus S-9 at the 250 µL dose were 1.04 and 0.91 respectively. Concentrates from two additional samples of the 4th Street Sewer (25/8-2 and 27/8-3,4) failed to elicit a mutagenic response in strain TA 100 with and without S-9. However, concentrate 27/8-3,4 caused a dose-related suppression in revertant numbers.

A test for DNA damage on concentrate 27/8-5,4 from a sample of the 4th Street Sewer was toxic to E.coli strain W3110 (Appendix XIII). Toxicity was not detected at the 100 µL dose on strain P3478 indicating the absence of DNA damaging activity.

A test for mutagenic activity on duplicate concentrates 29/5-2 and 3/6-2 from a sample of Steam Plant effluent failed to detect a mutagenic response in strains TA 98 and TA 100 with and without S-9 (Appendix IIIK).

Two influent water sources, the 3rd Street Service Water and the 4th Street Service Water were tested for mutagenic activity. Duplicate concentrates 27/5-3 and 2/6-3 of a sample from the 4th Street Service Water failed to induce a mutagenic response in tester strains TA 98 and TA 100 with and without S-9 (Appendix III I). Duplicate concentrates 29/5-1 and 3/6-3 from a sample of the 3rd Street Service Water also failed to elicit a mutagenic response.

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ORGANIC CHEMICAL ANALYSIS OF THE 2ND STREET, 3RD STREET AND 4TH STREET SEWER EFFLUENTS

Grab samples from the 2nd Street Sewer, 3rd Street Sewer and 4th Street Sewer were analyzed for volatile compounds, using GC/MS for identification and quantitation, by the Organic Trace Contaminants Section, Ministry of the Environment. The results of these analyses are given in Table 10. The compounds identified were reviewed on the basis of their ability to be positive in the Salmonella mutagenicity test. The compounds 1,1-dichloroethane and 1,2-dichloroethane, in their chemically pure form, had previously been detected as positive on strains TA 100 and TA 1535 in mutagenicity tests conducted in our laboratory(2). Methylene chloride, was scored presumptive mutagenic on TA 1535 (2) but because of the volatility of this compound, it is expected to be lost in tests employing the pour plate technique. The chemically pure form of the compounds chloroform, carbon tetrachloride, benzene,tetrachoroethylene, toluene, t-butanol and styrene were found negative in tests for bacterial mutagenicity (2).

Mutagenicity activity had previously been detected in 1,2,3,-trichloropropane (2). The results of a bacterial mutagenicity test on the chemical pure form of 1,2-dichloropropane are shown in Table 11.

The compound 1,2-dichloropropane, tested over a concentration range of 0.1 mg to 23 mg per plate, induced a dose-related increase in revertants in strain TA 100 and TA 100 plus S-9. The MUTAR value for this compound in TA 100 was 1.88. The presence of a dose-related increase in TA 100 and TA 100 and a MUTAR value exceeding 1.50 would indicate presumptive

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Table 10. Volatile organic compounds identified and quantified in effluent samples from Dow Chemical of Canada Limited.

Organic Compound	Effluent 2nd Street Sewer	Effluent 3rd Street Sewer	Effluent 4th Street Sewer
Compounds Quantified			
Methylene chloride	25.4*	0.8	0.5
1,1-dichloroethane	60.7	0.5	1.6
Chloroform	-	11.0	-
1,2-dichloroethane	-	-	4.0
Carbon tetrachloride	26.5	584	12.2
1,2-dichloropropane	2694	-	37.5
Benzene	3.1	1.0	2.4
Tetrachloroethylene	-	1.0	0.3
Toluene	-	61.3	0.3
Ethylbenzene	-	3.5	-
Additional Compounds Identified			
Butanol (tentative)	-	+	-
2-methyl-2-butanol	-	+	+
1,1-dichloropropane	+	-	-
Hexane	-	+	+
1,1,2-trichloroethane	-	-	+
Hexanol	+	-	-
Dichloropropene	+	-	-
4-vinyl-1-cyclohexene	-	+	+
Tetramethylbenzene	-	-	+
Styrene	-	+	-
Xylene	-	+	-
Bis(chloroisopropyl)ether	+	-	-
Bis(chloropropyl)ether	+	-	-

* concentration in ug/L
- not detected
+ detected

Table 11. Mutagenic activity of 1,2-dichloropropane in its chemically pure form.

	Revertants per plate			
	TA 98		TA 100	
	-S9	+S9	-S9	+S9
Solvent Control*	12	38	174	126
	13	35	129	102
Historical Average	15	28	145	103
Positive Controls				
MNNG[a] (2ug/plate)			5000	1455
2AF[b] (2ug/plate)		435		
2NF[c] (2ug/plate)	390			
1,2-dichloropropane				
23 mg[d]	12	22	424	282
11.5mg	17	17	284	161
1.2mg	15	40	192	83
0.1mg	14	32	176	124

* DMSO at 100uL/plate
a N-methyl-N'-nitro-N-nitrosoguanidine
b 2-aminofluorene
c 2-nitrofluorene
d 1,2-dichloropropane diluted to give the desired concentration in 100uL DMSO.

mutagenic activity in the chemically pure form of the compound.

SUNOCO INCORPORATED

The final effluent of Sunoco Incorporated was tested for mutagenic activity and DNA damaging activity. The results of the mutagenicity test on concentrates of this effluent are given in the Appendix IV and these results are summarized in Table 12. The results of a test for DNA damaging activity on this effluent are given in the Appendix XIII and these results summarized in Table 13.

The responses induced by concentrate 6/5-2 from the final effluent in strains TA 98 and TA 100 with and without S-9 were within the variability of their respective solvent controls and these responses were considered non-mutagenic(Appendix IV).

Concentrate 21/1-2, from the final effluent also failed to elicit a mutagenic response in strain TA 98 with and without S-9. A 100 µL dose of 21/1-2 induced a response in TA 100 which exceeded revertant numbers in the solvent control by a factor of 0.73. In TA 100 plus S-9, the response at the three doses of 21/1-2 was within the variability of revertant numbers in the solvent control. The response in TA 100 at the 50 and 200 µL doses of this concentrate were similar to revertant counts in the solvent control. It was assummed that the response at the 100 µL dose was within the variability of the test and was not an indication of mutagenicity.

Concentrate 9/1-1 from a sample of the Sunoco Incorporated final effluent was not toxic at 100 µL dose to E. coli strains W3110 and P3478(Appendix XIIII). DNA damaging activity was not detected in this concentrate.

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Table 12. Summary of the results of the Salmonella mutagenicity test on concentrated samples of industrial effluents

Industry	Concentrate Number	Mutagenicity Score TA 98 -S9	TA 98 +S9	TA 100 -S9	TA 100 +S9
Sunoco Incorporated					
Final Effluent	6/5-2	-	-	-	-
	21/1-2	-	-	-	-
Shell Canada Limited					
Final Effluent	8/5-2	-	-	-	-
Contaminated Water	13/5-1	-	-	-	-
Ethyl Corporation of Canada Limited					
Final Effluent	12/5-1	-	-	-	-
	26/3-1	-	-	(-)	-
	15/1-1	(+)	(-)	+	+
Dupont Canada Incorporated					
Final Effluent	7/5-2	-	-	-	-
Canadian Industries Limited					
Final Effluent	7/5-3	-	-	-	-
Petrosar Limited					
Final Effluent	5/5-2	-	-	-	-

Table 13. Summary of the results of the E. coli test for DNA damaging activity on concentrated samples from industrial effluents.

Industry	Concentrate Number	Toxicity	DNA Damage
Sunoco Incorporated			
Final Effluent	9/1-1	-	-
Ethyl Corporation of Canada Limited			
Final Effluent	15/1-1	-	-
	29/1-5	-	-
Dupont Canada Incorporated			
Final Effluent	10/1-1	-	-
	24/1-3	-	-
Canadian Industries Limited			
Final Effluent	21/1-3	-	-

SHELL CANADA LIMITED

Concentrates of samples of the final effluent and of contaminated water from Shell Canada Limited were tested for mutagenic activity. The results of the mutagenicity test on these concentrates samples are given in the Appendices VA and VB and are summarized in Table 12.

Concentrate 12/5-1 of a sample of contaminated water failed to elicit a mutagenic response in tester strains TA 98 and TA 100 with and without S-9 (Appendix VB).

A concentrate from a sample of the final effluent 8/7-2 also failed to induce a mutagenic response in TA 98 and in TA 100 with and without S-9 (Appendix VA). In TA 98 plus S-9 and at 75 µL dose of 8/5-2, revertant numbers exceeded the variability of those in the solvent control. This increase in TA 98 revertant numbers at only one dose of this concentrate was considered insufficient evidence to conclude a mutagenic response in this concentrate.

ETHYL CORPORATION OF CANADA LIMITED

Concentrated samples from the final effluent of Ethyl Corporation of Canada Limited were tested for mutagenic activity and DNA damaging activity. The results of the mutagenicity test are given in the Appendix VI and these results are summarized in Table 12. The results of tests for DNA damaging activity on concentrates of this effluent are given in the Appendix XIII and these results are summarised in Table 13.

Concentrate 15/1-1 from the final effluent was tested for mutagenic activity at two doses, 50 and 100 µL, and elicited

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a dose-related increase in revertant numbers in tester strains TA 98 and TA 100 with and without S-9 (Appendix VI). In TA 98 and TA 98 plus S-9, revertant numbers induced by the 100 µL dose of 15/1-1 exceed those of the solvent control by a factor of 1.75 and 1.58 respectively (MUTAR 2.15 and 1.26 respectively). In TA 100 and TA 100 plus S-9, revertant numbers at the 100 µL dose of 15/1-1 exceed those of their respective solvent controls by a factor of 2.98 and 4.15 respectively (MUTAR 3.69 and 4.89 respectively).

Concentrate 26/3-1 of a second sample of this effluent failed to elicit a mutagenic response in TA 98, TA 98 plus S-9 and TA 100 plus S-9. However, at doses ranging from 19 to 75 µL, this concentrate induced a dose-related increase in revertants in TA 100. At the 75 µL dose, TA 100 revertant numbers exceeded those of the solvent control by a factor of 0.47 (MUTAR = 0.58).

Concentrate 12/5-1 from a third sample of this effluent failed to induce a mutagenic response in tester strains TA 98 and TA 100 with and without S-9.

Concentrates 15/1-1 and 29/1-5 from this final effluent were not toxic in the E. coli test for DNA damage (Appendix XIII). DNA damaging activity was not detected in concentrates 15/1-1 and 29/1-5.

DUPONT CANADA INCORPORATED

The final effluent at Dupont Canada Incorporated was tested for mutagenic activity and DNA damaging activity. The results of tests for mutagenic activity are given in the Appendix VIII and these results are summarized in Table 12. The results of tests for DNA damaging activity are given in the Appendix XIII and these results are summarized in Table 13.

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Concentrate 7/5-2 from the final effluent failed to elicit a mutagenic response in tester strains TA 98 and TA 100 with and without S-9 (Appendix VII). DNA damaging activity in E. coli was not detected in two additional concentrated samples (10/1-1 and 24/1-3) from this effluent.

CANADIAN INDUSTRIES LIMITED

A concentrated sample from the final effluent of Canadian Industries Limited failed to induce a mutagenic response in tester strains TA 98 and TA 100 with and without S-9 (Appendix VIII). A test on an additional concentrated sample of this effluent (21/1-3) was not toxic in E. coli tester strains W3110 and P3478 and at a 100 µL dose did not contain DNA damaging activity (Appendix XIII).

PETROSAR LIMITED

The results of the mutagenicity test on a sample from the Petrosar Limited final effluent are given in the Appendix IX and these results are summarized in Table 12.

An increase in revertant numbers in TA 98 and a suppression in revertant numbers in TA 100 was induced with increasing doses of concentrate 5/5-2 (Appendix IX). The responses in TA 98 and TA 100 were, however, within the variability of revertant numbers in their respective solvent controls. Concentrate 5/5-2 from the final effluent also failed to elicit a mutagenic response in TA 98 plus S-9 and TA 100 plus S-9.

MUTAGENICITY TESTING OF ST. CLAIR RIVER SAMPLES

Water samples were taken from five stations in the St. Clair River. The location of the sampling stations are

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Table 14. Documentation of locations and the sampling dates of river samples taken from the St. Clair River downstream of Sarnia, Ontario.

River Station	Date Sampled	Station Description
Upstream of the Township Ditch	July3,1980	In the river, 30m offshore and 45m upstream of the Township Ditch Effluent at Polysar Limited.
Downstream of the Township Ditch and Upstream of the Dow 1st Street Complex	July3,1980	Downstream of the Township Ditch, 15m offshore and opposite the northern property fence of Dow Chemical of Canada Limited.
In the plume of the Dow 1st Street Complex	July3,1980	In the effluent plume, 5m offshore and 20m downstream of the Dow Chemical of Canada Limited, 1st Street effluent complex.
In the plume of the Dow 2nd Street Sewer	July3,1980	In the effluent plume, 10m offshore and 20m downstream of the Dow Chemical of Canada Limited, 2nd Street Sewer discharge.
Downstream of the Dow 4th Street Sewer	July3,1980	In the river, 15m downstream of the 4th Street Sewer discharge and midway between the shore and the loading dock of Dow Chemical of Canada Limited. The sewer outfall was surrounded by a floating boom.

described in Table 14. The volatile organic fraction of four litre samples was concentrated into a final concentrate volume of 2.4 mL. The results of the mutagenicity test on these concentrated samples are given in the Appendix XIV through XIV and these results are summarized in Table 15.

A concentrated sample from a river station upstream of the Township Ditch failed to elicit a mutagenic response in all tester strains (Appendix XIVA).

Concentrate 14/7-2, obtained from a river station downstream of the Township Ditch and upstream of Dow Chemical of Canada Limited (Appendix XIVB) and a concentrated sample from a river station within the effluent plume of the Dow Chemical of Canada 1st Street effluent complex also failed to induce a mutagenic response (Appendix XIVC).

Duplicate concentrates 9/7-1 and 9/7-2 from a river station within the effluent plume of the Dow Chemical of Canada 2nd Street Sewer were tested for mutagenic activity (Appendix XIVD). Both concentrates failed to induce a response in TA 100 and TA 98.

In TA 100 plus S-9, both concentrates 9/7-1 and 9/7-2 elicited a response which increased with increasing dose of concentrates. At the 250 µL dose of 9/7-1 only, revertant numbers in TA 100 plus S-9, exceeded the variability in the solvent control and exceeded revertant numbers in the solvent control by a factor of 0.88. The response induced by these duplicate concentrates in TA 100 and TA 100 plus S-9 was of insufficient magnitude to conclude a mutagenic response.

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Table 15. Summary of the results of the Salmonella mutagenicity test on concentrated samples of St. Clair River water

River Station	Concentrate Number	Mutagenicity Score TA 98 -S9	TA 98 +S9	TA 100 -S9	TA 100 +S9
Upstream of the Township Ditch	11/7-1	-	-	-	-
Downstream of the Township Ditch and Upstream of the Dow 1st Street Complex	14/7-2	-	-	-	-
In the plume of the Dow 1st Street Complex	8/7-1	-	-	-	-
In the plume of the Dow 2nd Street Sewer	9/7-1	-	-	-	-
	9/7-2	-	-	-	-
Downstream of the Dow 4th Street Sewer	10/7-1	-	-	-	-

In TA 98 plus S-9, and at the 31 µL dose of concentrate 9/7-2, revertant numbers exceeded those of the solvent control by a factor of 1.83. At the 31 µL and 63 µL doses of duplicate concentrate 9/7-1, revertant numbers of TA 98 with S-9 exceeded those of the solvent control by a factor of 0.97 and 1.14 respectively. The response in TA 98 plus S-9,particularly at the 31 µL dose in both concentrates, suggests a dose-response in this sample. However, considering the variability observed with TA 98 plus S-9 in other concentrated samples, this response is not considered of sufficient magnitude to conclude TA 98 mutagenic activity in this sample.

A concentrated sample (10/7-1) from a river station downstream of the Dow Chemical of Canada 4th Street Sewer failed to elicit a mutagenic response in tester strains TA 98 and TA 100 with and without S-9 (Appendix XIVE). The response of this concentrate in TA 98 and TA 98 plus S-9 was also within the variability of the solvent control.

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DISCUSSION

The objective of this study was to test for genotoxic (genetic damaging) activity in the volatile organic component of industrial effluents entering the St. Clair River. This study utilized two short-term genotoxicity tests: a modification of the Salmonella mammalian microsomal mutagenicity test and a modification of the Rosenkranz Pol A/Pol A$^-$ test for DNA damage in Escherichia coli. The aim of the study was to use these bacterial tests to identify effluents which contain mutagenic and/or DNA damaging activity.

A study of this type has inherent problems. One major difficulty may occur in the application of these tests to samples which were known to contain mixtures of organic compounds. The reason for this concern was that these tests were developed and evaluated using chemically pure compounds, and not complex mixtures. The effects on the test organism of the multitude of interactions within an organic mixture could lead to toxicity, as well as positive or negative synergistic reactions. These potential interactions were expected to pose difficulties in the detection of a genotoxic response. Mutagenic and/or DNA damaging activity was detected in concentrated effluent samples, thus suggesting these tests to be capable of identifying this activity in organic mixtures. These positive results do not preclude the possibility that the above mentioned interactions may have masked the genotoxic activity in other concentrates.

A second major difficulty concerns the ability of these tests to detect genotoxic agents at the organic chemical concentrations expected in these effluent samples. Furthermore, the identification of mutagenic activity requires the testing of the agent at concentrations above the detection limit in order

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to demonstrate a dose-related response. A concentration step was therefore necessary to bring levels of organic compounds within the measurable range of these tests.

A concentration method was developed to selectively concentrate the volatile organic compounds in industrial effluents. Other compounds, such as polar organic compounds were not recovered by the concentration procedure and consequently were not included in the concentrates tested. Conclusions regarding the presence or absence of genotoxic activity in concentrated samples pertain only to the recovered volatile organic component.

Because of a limitation in the quantity of concentrated samples, mutagenicity testing was restricted to strains TA 98 and TA 100. The omission of tester strains TA 1535 and TA 1538 was considered justified since strains TA 100 and TA 98 respectively detect similar and theoretically additional mutagenic compounds. The Salmonella strains employed in this study would not detect those compounds mutagenic only in strain TA 1537. A previous study on chemically pure forms of compounds identified in the St. Clair River system, included tester strain TA 1537 and of those volatile compounds tested, none were mutagenic to TA 1537(2).

The amount of the concentrated sample available restricted the mutagenicity testing to three or four doses of concentrates and one plate per dose per tester strain. The response of the tester strain of three or four doses of the concentrated sample was generally sufficient to conclude a dose-related response.

A testing of a single plate per dose provided a measurement of the response of the tester strain but did not

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supply information on the variability of this response. Variability within the tester strain was calculated from solvent control plates in triplicate. A response at a particular dose was considered different from the solvent control value if the revertant numbers at that dose exceeded the 95% confidence limit (n=3) of the standard deviation of revertant numbers in the solvent control.

The organic composition of an industrial effluent changes continuously. These fluctuations in effluent composition pose serious problems in detecting the possible presence of genotoxic activity in a particular industrial effluent. To facilitate better recovery of the volatile organic compounds a grab sampling technique was used in this study. Such a sampling technique is only representative of the effluent at the time of sampling. Interpretations of the results of genotoxicity tests must be viewed as the presence or absence of genotoxic activity in an effluent at the time of sampling and cannot be used to predict the effluent quality over an extended time period.

Testing of duplicate concentrates of an effluent sample and the resampling of an efflunt were used in this study to confirm the presence or absence of mutagenic activity. Confirmation of a test result was best achieved when duplicate concentrates of the same effluent sample were tested. In those cases where mutagenic activity was detected in a concentrated effluent sample and this activity was confirmed in a duplicate concentrate of the same sample, then the presence of mutagenic activity was concluded for that sample. In the case where mutagenic activity was detected in a concentrated sample but a duplicate concentrate

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of that sample was not tested to confirm this activity, then a second sample of the effluent was tested. If mutagenic activity was also detected in this additional sample, then it was concluded that mutagenic activity was present in the effluent. In one case, mutagenic activity was detected only in the initial sample of the effluent. Two additional samples from this effluent failed to elicit a mutagenic response. It was assummed that the discrepancies in the results of the mutagenicity tests on this effluent were due to variability in effluent chemical composition.

Although this positive result cannot be ignored, it was not possible to confirm this activity and therefore no definite conclusions as to the presence of mutagenic activity could be made.

In those cases where mutagenic activity could not be detected in any of the concentrated samples from an effluent, then it was assumed that the effluent did not contain mutagenic activity. It was possible to conclude the absence of mutagenic activity in those effluent samples tested. It was not possible to predict however, that mutagenic activity would not have been detected if larger samples or additional samples were taken at different times from these effluents.

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CONCLUSIONS

Effluent samples from nine industries situated along the St. Clair River downstream of Sarnia, Ontario, were studied. The concentrated volatile component from a total of 25 effluents from these industries as well as 5 influent sources were tested.

Concentrated samples from 21 effluents and one influent source were tested for mutagenic and/or DNA damaging activity. Mutagenic activity and DNA damaging activity were detected in samples from one effluent. Mutagenic activity was detected and confirmed in samples from an additional two effluents and detected in a third effluent. Samples from an additional three effluents induced DNA damage.

Four effluents and four influent sources were tested only for mutagenic activity. All samples from these four effluents as well as the four influents failed to elicit a mutagenic response.

The conclusions given to the results of these tests on specific effluents are given below.

Imperial Oil Enterprises Limited

Mutagenic activity was not detected in the five effluents tested. A sample from the Pressure Sewer final effluent was toxic to tester strains TA 98 and TA 100 and to *Escherichia coli*. This sample also induced DNA damaging activity.

Polysar Limited

Mutagenic activity was not detected in the Township Ditch effluent and was also not detected in the 54" Sewer, 66" Sewer, 72" Sewer and Stereo API effluents from Polysar Limited.

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A concentrated sample from each of two effluents, the Stereo API and the 72" Sewer, was toxic to TA 100. The Township Ditch influent and the Service Water influent samples were negative in the mutagenicity test. One sample from the Stereo API effluent induced DNA damaging activity. All remaining effluents tested failed to induce DNA damaging activity and this activity was not detected in either influent sources.

Dow Chemical of Canada Limited

Samples from two influent sources, namely the 3rd Street Service Water and the 4th Street Service Water, failed to elicit a mutagenic response. Effluent samples from the 42" Sewer, 48" Sewer, 54" Sluice, and the Steam Plant were also negative in the mutagenicity test. One concentrated sample from the 54" Sewer demonstrated toxicity in TA 100.

Samples of the 3rd Street Sewer demonstrated toxicity, as indicated by a clearing of the bacterial lawn and in the dose-related reduction in the number of revertants for TA 100. However, toxicity to E. coli was not observed. Mutagenic activity and DNA damage were not detected in any samples from this effluent.

Duplicate concentrates of a sample from the 2nd Street Sewer induced a dose-related increase in revertant numbers in TA 100 with and without S-9. An additional concentrated sample from this effluent also elicited a mutagenic response in both concentrates from one sample and in an additional sample of this effluent. The presence of mutagenic activity was concluded. Furthermore, DNA damaging activity was detected in a sample of this effluent and from these results we conclude the presense of genotoxic activity in this effluent.

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Three samples of the 3rd Street Sewer demonstrated toxicity to TA 100. Mutagenic activity and DNA damaging activity was not detected in this effluent.

A concentrate (28/8-9,10) from an initial sample of the Direct Oxidation of Ethylene Oxide (DOEO) effluent was toxic to TA 100 both with and without S-9. This concentrate at a dose of 50 µL induced revertant numbers in TA 100 which exceeded the spontaneous revertant level by a factor of 0.94, and which exhibited a MUTAR value of 0.90. It was assumed that the elevated revertant numbers induced by the initial sample was an indication of mutagenic activity. This response in TA 100 could not be confirmed in subsequent samples taken from this effluent. Furthermore, neither toxicity in E. coli nor DNA damaging activity was observed in concentrate 28/8-9,10. This absence with E. coli of DNA damaging activity neither supports or precludes the presence of mutagenic activity in this concentrated effluent sample. For these reasons no definite conclusion as to the mutagenic activity of this effluent could be made.

Duplicate concentrates of a sample from the 4th Street Sewer demonstrated a dose-related increase of revertants in TA 100 with and without S-9. Based on this duplication of a mutagenic response it was concluded that this sample contained mutagenic activity. DNA damaging activity was not detected in this effluent. However, toxicity to Salmonella and to E. coli W3110 was detected in an additional sample.

Chemical analysis of the volatile organic component from the 2nd and 4th Street Sewers identified methylene chloride 1,1-dichloroethane, 1,2-dichloroethane and 1,2-dichloropropane.

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All of these compounds, in their chemically pure form, have been found, in this and in a previous study(2), to induce a positive mutagenic response in strain TA 100 with and without S-9. At this stage of the study, one cannot attribute the mutagenic activity observed in samples from the 2nd and 4th Street Sewers directly to any of these compounds. Nevertheless, these mutagenic compounds may have contributed, at least in part, to the observed mutagenic activity in these effluent samples.

Sunoco Incorporated

Concentrated samples from the final effluent of Sunoco Incorporated failed to elicit a mutagenic response. DNA damaging activity was not detected in an additional sample of this effluent.

Shell Canada Limited

Mutagenic activity was not detected in concentrated samples of the final effluent and contaminated water from Shell Canada Limited.

Ethyl Corporation of Canada Limited

Mutagenic activity, as indicated by an a) dose-related increase in revertants of TA 98 and TA 100 with and without S-9 and b) MUTAR values in excess of 2.5 in TA 98, TA 100 and TA 100 plus S-9, was detected in one effluent sample of the Ethyl Corporation of Canada. A mutagenic response in TA 100 with a MUTAR value of 0.58 was detected in an additional sample of this effluent. The presence of mutagenic activity in this effluent was confirmed. DNA damaging activity was not detected in the final effluent from this industry.

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Dupont of Canada Limited

Bacterial mutagenic activity and DNA damaging activity was not detected in concentrates of the final effluent from Dupont of Canada Limited.

Canadian Industries Limited

The final effluent of Canadian Industries Limited failed to induce mutagenic activity in the Salmonella test and failed to elicit DNA damaging activity in E. coli.

Petrosar Limited

A concentrated sample of the final effluent of Petrosar Limited failed to induce a mutagenic response in Salmonella.

St. Clair River Samples

The mutagenic activity in the volatile organic component of St. Clair River water was tested on concentrates from four litres of sample. This theoretically affords a four-fold increase in concentration of volatile organic compounds compared to that obtained from effluent samples.

River samples taken from a) upstream of the Township Ditch, b) downstream of the Township Ditch and upstream of Dow Chemical of Canada, c) within the Plume of the Dow 1st Street Complex and d) downstream of the Dow 4th Street Sewer were tested. Samples from these four river stations failed to induce a mutagenic response. Duplicate concentrates of a river sample taken within the plume of the Dow 2nd Street Sewer demonstrated a dose-related increase in revertants in TA 100 plus S-9. This response was generally within the variability of the revertant count in the solvent control but in one concentrate at the maximum dose of 250 µL TA 100 revertants exceeded this variability

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limit. At this dose, a MUTAR value of 0.6 was calculated. It was not possible to conclude mutagenic activity in this sample. The response to this sample in TA 100 plus S-9 may be attributed to natural variation in the spontaneous revertant levels in TA 100. The testing of a concentrate prepared from a volume of sample larger than four litres would be necessary to confirm the absense of mutagenic activity in this river sample.

The results of this study demonstrated that an analytical procedure, incorporating a concentration method for volatile organic compounds and using modifications of the Ames and Rosenkranz tests, was capable of detecting mutagenic and/or DNA damaging activity in industrial effluents.

The detection of genotoxic activity in some industrial effluents must be interpreted carefully. The tests employed in this study are bacterial and only at the detection stage of an evaluation for a potential genotoxic hazard. A complete evaluation of such a hazard requires the results of a more extensive study incorporating tests in mammalian cells. The genotoxicity tests used in this study were applied to a mixture of volatile organic compounds and this mixture was tested at concentrations considerably higher than present in the effluent samples. Moreover, levels of these compounds originally present in these effluents would be greatly diluted in the river. An evaluation of the fate of mutagenic compounds in the environment, including their persistence, bioaccumulation and biodegredation is necessary before a full assessment of a genotoxic hazard can be made. Finally, the results of short-term genotoxicity tests,

including verification tests, are insufficient in defining risk. An assessment of risk would require more extensive animal mutagenicity and carcinogenicity studies. The results of this report are best used in the setting of priorities for a more extensive investigation of a possible genotoxic hazard in industrial effluents.

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RECOMMENDATIONS

1. An analytical approach utilizing short-term tests for mutagenicity and DNA damage and assisted by a concentration method for volatile organic compounds has been found effective in detecting genotoxic activity in industrial effluents. Such an approach is a potential tool for the testing of the quality of industrial and municipal effluents.
2. The detection of mutagenic and DNA damaging activity in industrial effluents indicates a possible source of genotoxic hazard to the environment from certain effluents. This finding should initiate the screening of other industrial and municipal effluents for genotoxic activity.
3. Chemical analyses of industrial effluents have identified a series of volatile organic compounds. Tests on the chemically pure forms of some of these compounds, have detected mutagenic activity. The fate of these mutagenic compounds in the environment should be investigated. These investigations should include a study of the environmental persistence, bioaccumulation and biodegradation of these compounds in order to more accurately assess their hazard to the environment and ultimately to man.
5. The concentration procedure employed in this study was designed for the selective concentration of volatile organic compounds. The development of concentration methods to permit the genotoxic testing of additional components of the organic matrix in industrial effluents is required.

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6. The short-term bacterial tests employed in this study are at the detection level of a screen for genotoxic activity. The development of a battery of short-term genotoxicity tests incorporating mammalian systems is necessary for the verification of this genotoxic hazard.

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APPENDICIES I THROUGH IX

Appendices I through IX contain the results of the mutagenicity test on concentrated samples of industrial effluents. This mutagenicity test used the Ames tester strains Salmonella typhimurium TA 98 and TA 100 in the presence and absence of metabolic activation (S-9). These samples and tester strains were combined using the pour plate technique.

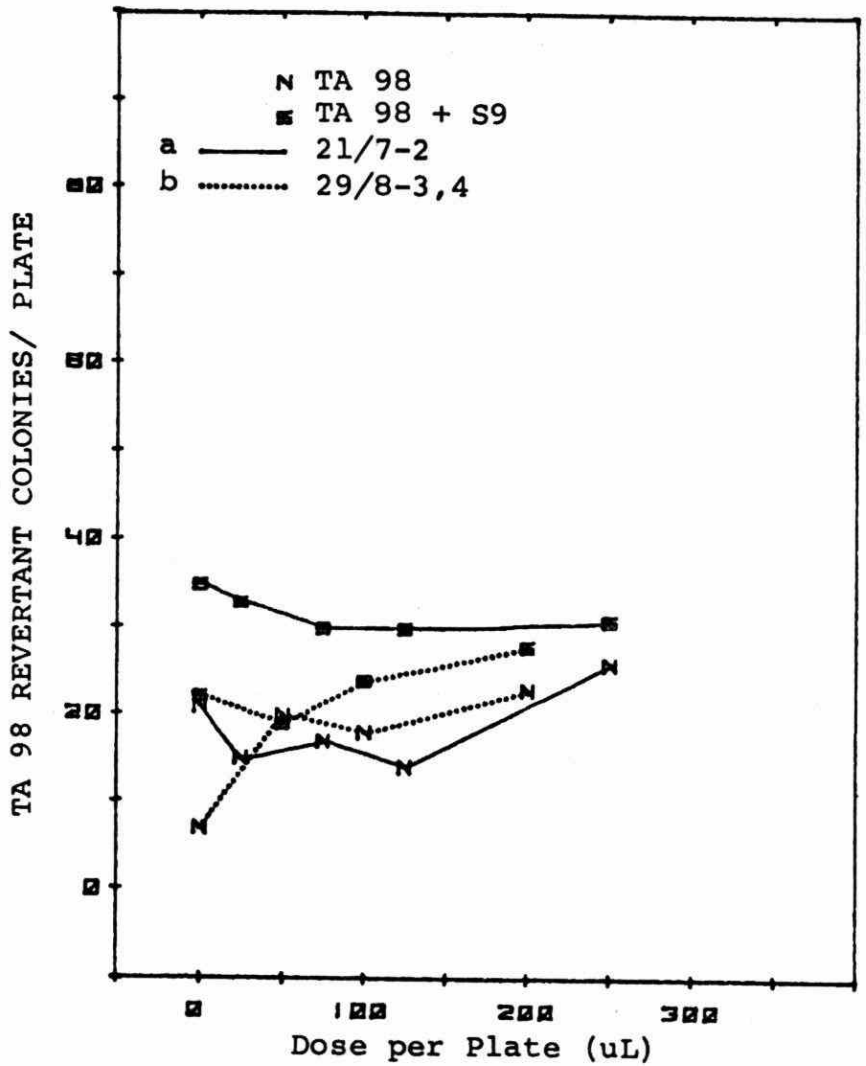
The results of these tests on concentrated samples of industrial effluents were expressed as a plot of revertant numbers per plate against dose (μL) of concentrated sample. The results of these tests in strain TA 98 with and without S-9 were generally plotted in the left hand figure, while the results of those tests in strain TA 100 and TA 100 plus S-9 were generally plotted in the right hand graph. In cases where more than three concentrated samples were tested per effluent, then the results of tests in TA 98, plus S-9, TA 100 and TA 100 plus S-9 were plotted in independent figures.

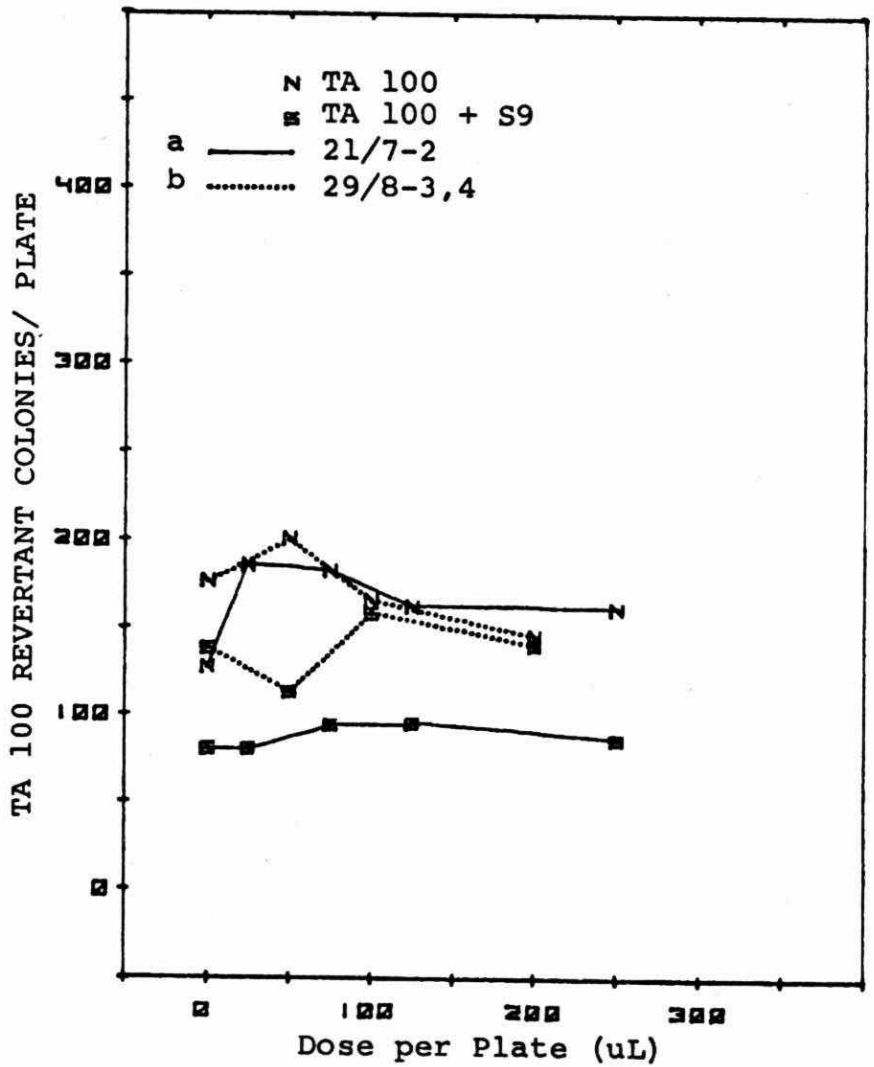
Concentrated samples from an industrial effluent were identified in the figure by their concentrate number and were differentiated in the plot by the characteristic of the line. Data points for the response of strains TA 98 and TA 100 to a particular dose of the concentrated sample, in the absence of S-9, were denoted by the symbol "N". Data points of the response to these doses by strain TA 98 and TA 100 in the presence of S-9 were denoted by the symbol "S".

Background revertant numbers for each strain were calculated for each test by averaging revertant numbers on three solvent control plates. Solvent control revertant numbers are presented in the following figures by the response at zero dose

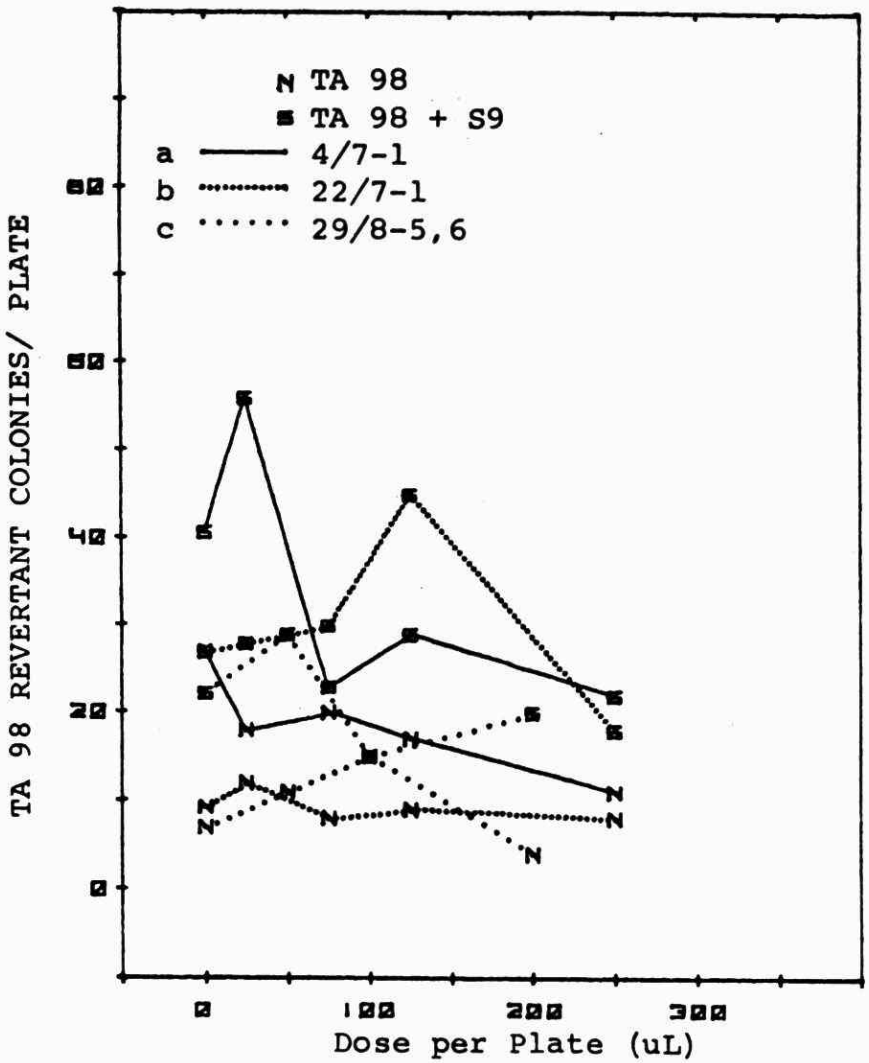
of the concentrated sample.

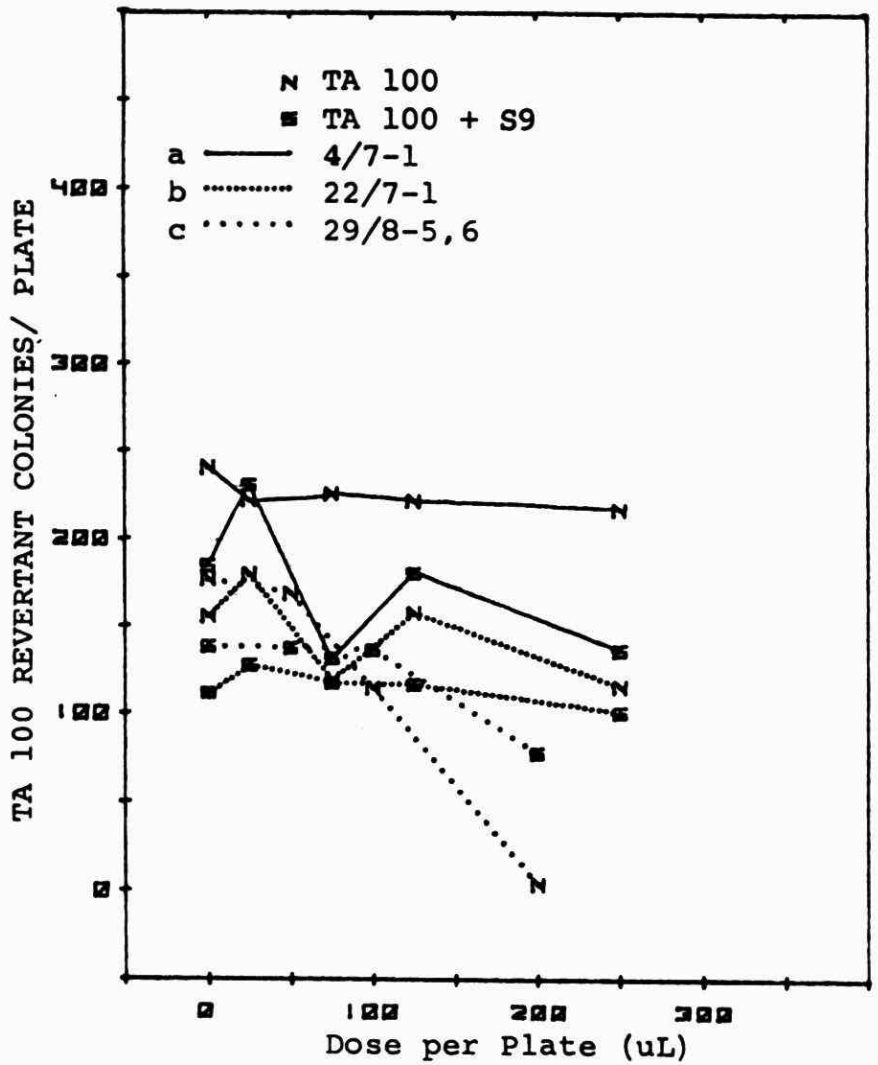
The response of the tester strain to known mutagenic compounds (positive controls) is presented in the text of the figures.



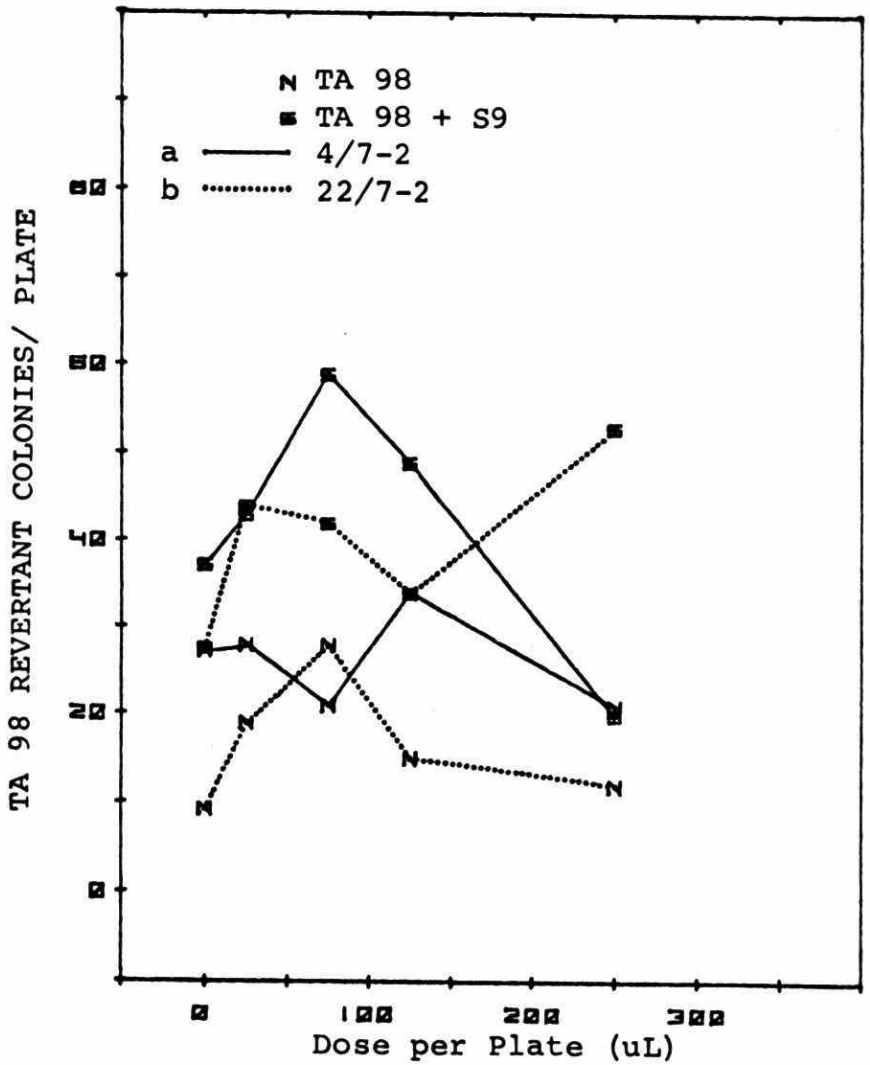


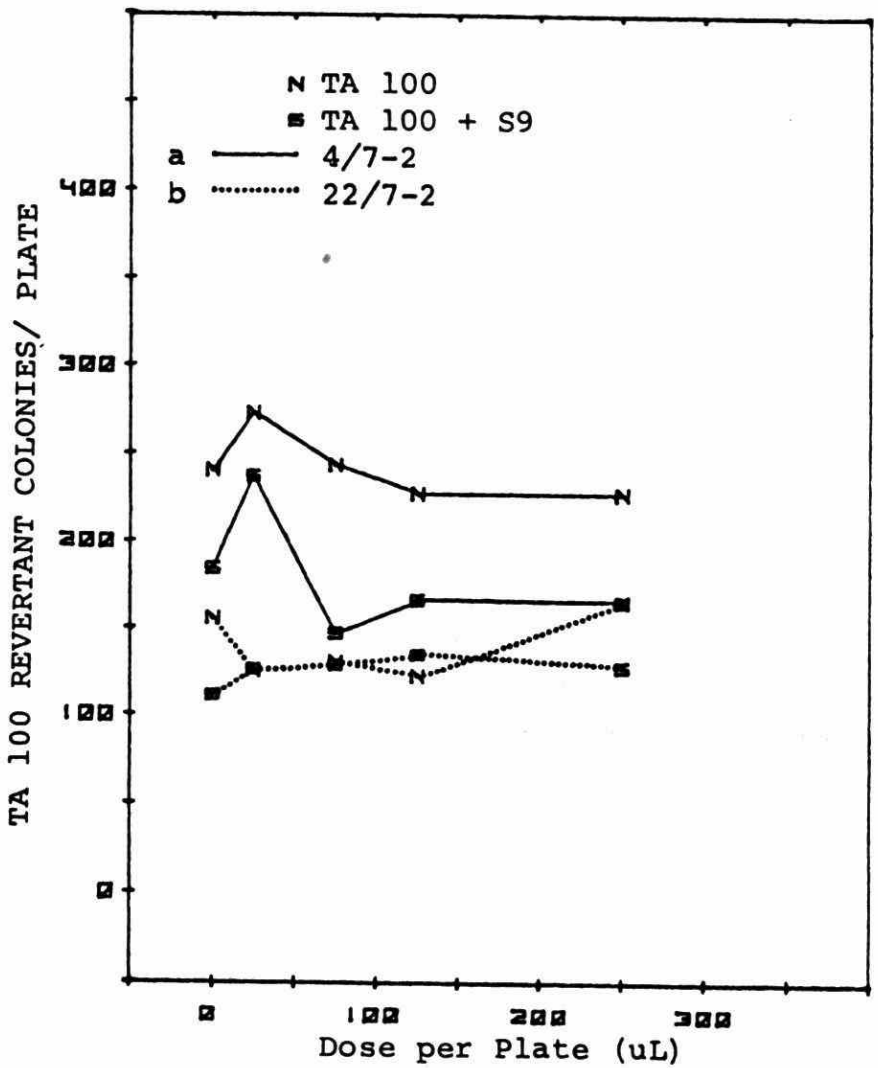
Appendix I A. Bacterial mutagenic response to the # 3 Separator effluent concentrate of Imperial Oil Enterprises Limited.
Positive controls TA 98 + S9 and 2AF; a) 1593 @2ug, b) 2400 @5ug
TA 100 and MNNG a) 451 @2ug, b) 6240 @10ug



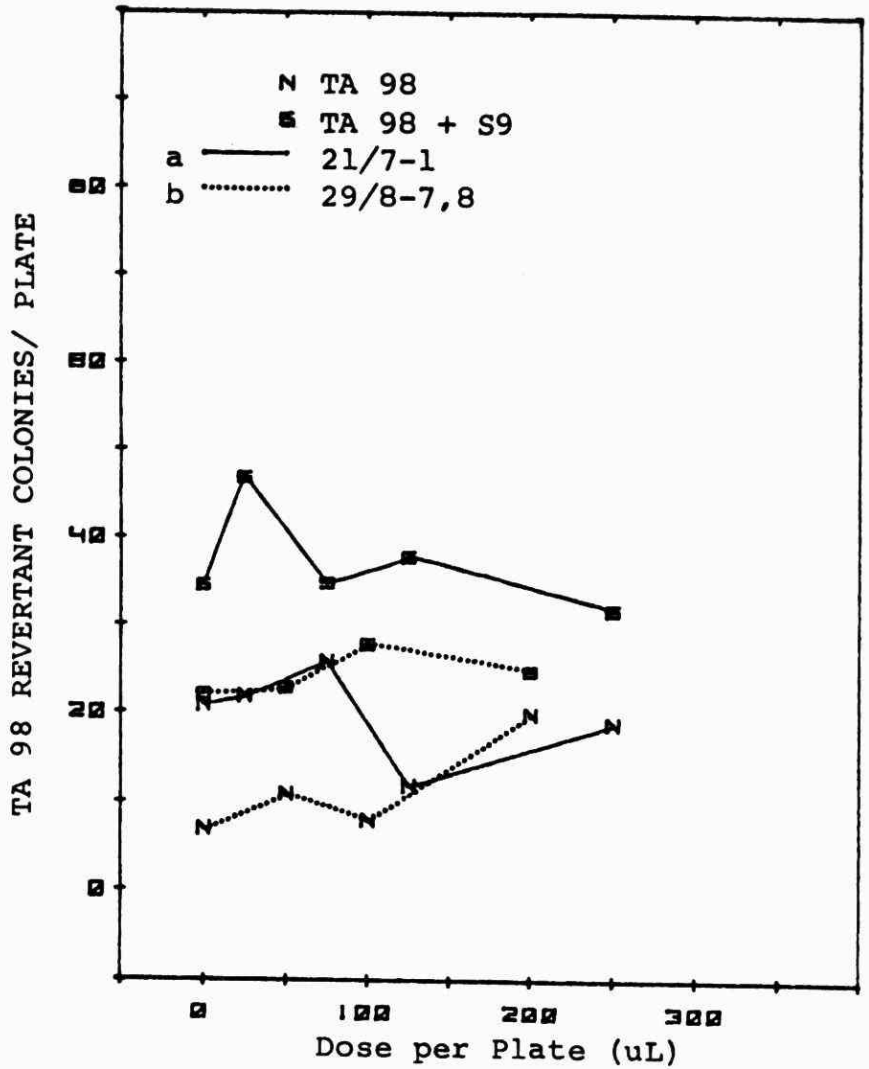


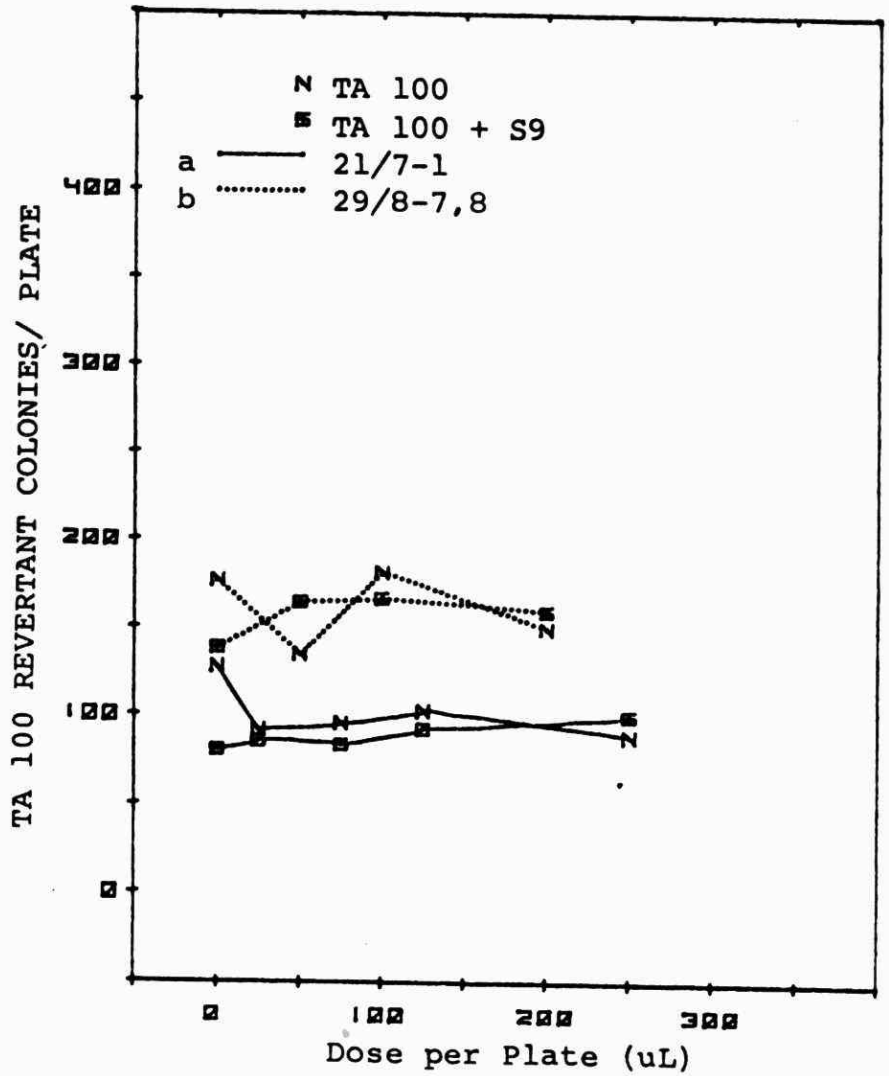
Appendix I B. Bacterial mutagenic response to the Pressure Sewer's Final effluent concentrate, Imperial Oil Enterprises Limited.
Positive controls: TA 98 + S9 and 2AF; a) 595 @2ug, b) 152 @2ug, c) 2400 @5ug
TA 100 and MNNG; a) 2240 @2ug, b) 1365 @2ug, c) 6240 @10ug



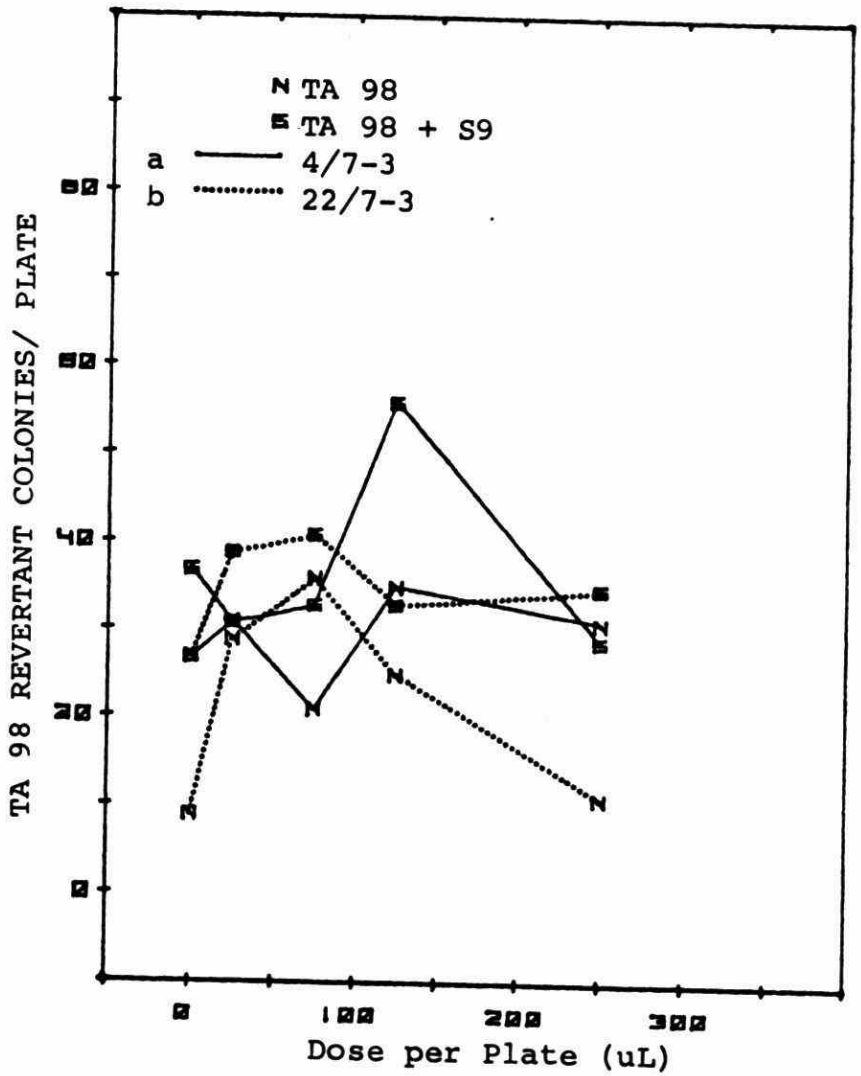


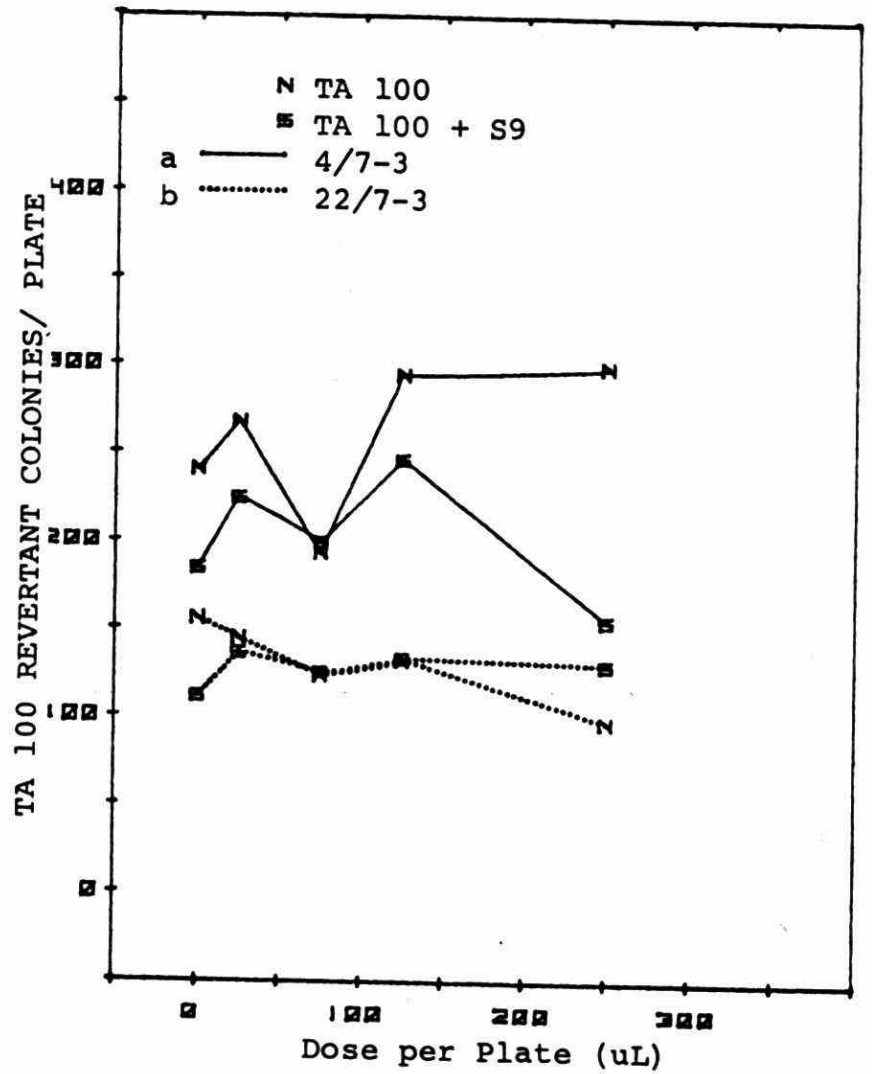
Appendix I C. Bacterial mutagenic response to the # 9 Separator effluent concentrate of Imperial Oil Enterprises Limited.
Positive controls: TA 98 + S9 and 2AF; a) 595 @2ug, b) 152 @2ug
TA 100 and MNNG; a) 1545 @2ug, b) 1870 @2ug



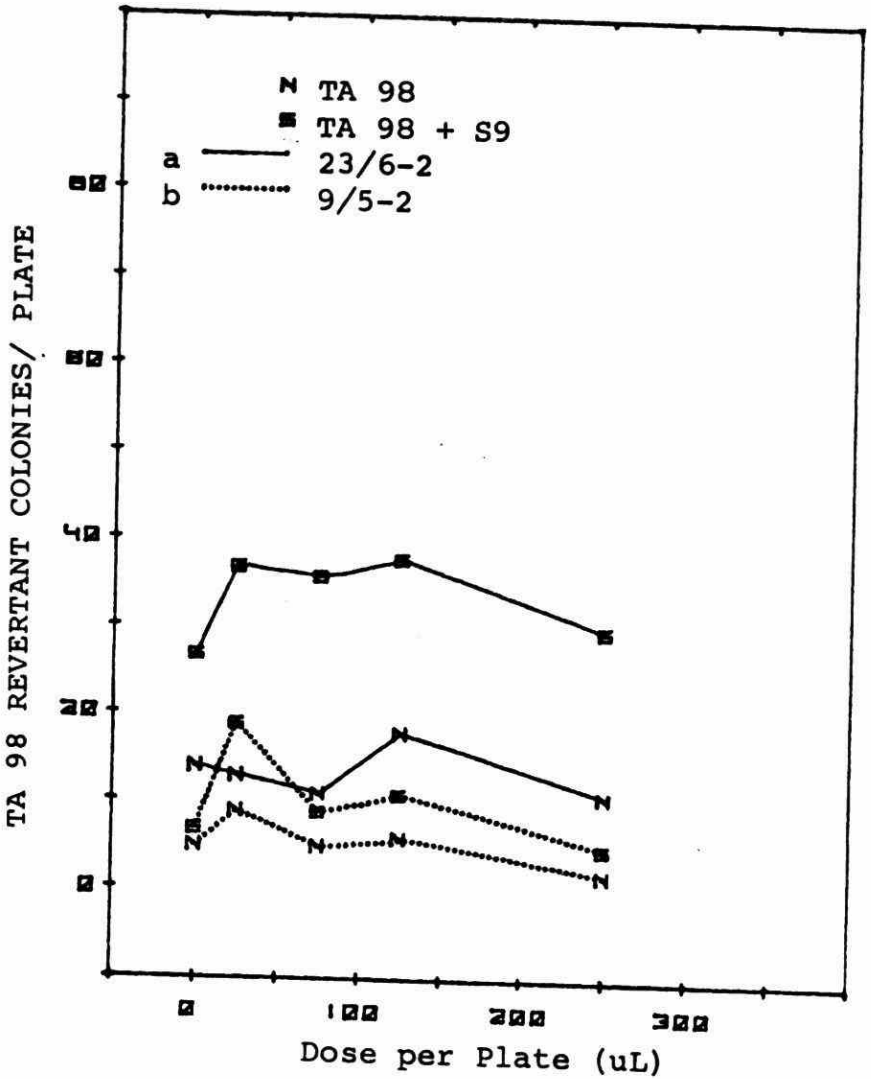


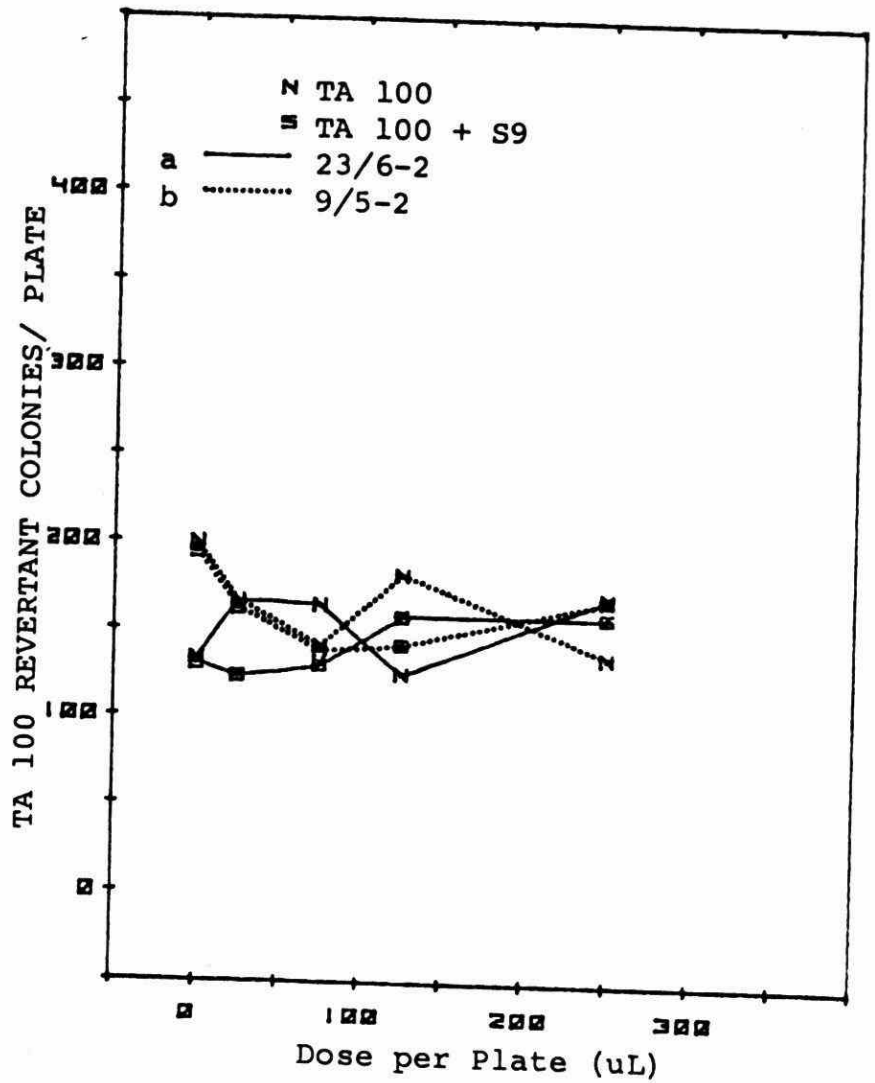
Appendix I D. Bacterial mutagenic response to the Biooxidation system effluent concentrate of Imperial Oil Enterprises Limited.
Positive controls: TA 98 + S9 and 2AF; a) 1593 @2ug, b) 2400 @5ug
TA 100 and MNNG; a) 451 @2ug, b) 6242 @10ug



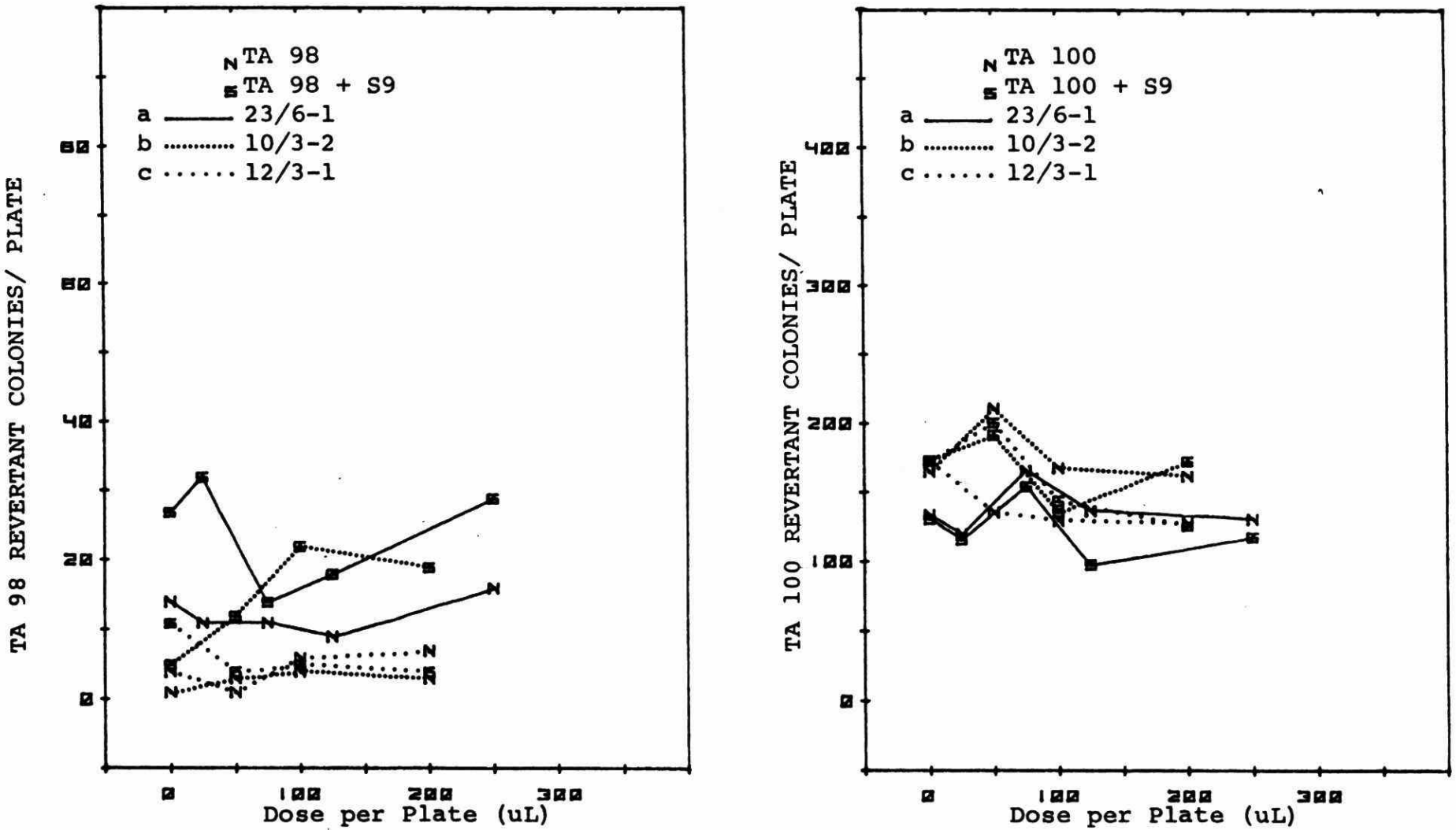


Appendis I E. Bacterial mutagenic response to the Service water concentrate of Imperial Oil Enterprises Limited.
Positive controls: TA 98 + S9 and 2AF; a) 595 @2ug, b) 152 @2ug
TA 100 and MNNG; a) 1545 @2ug, b) 1870 @2ug

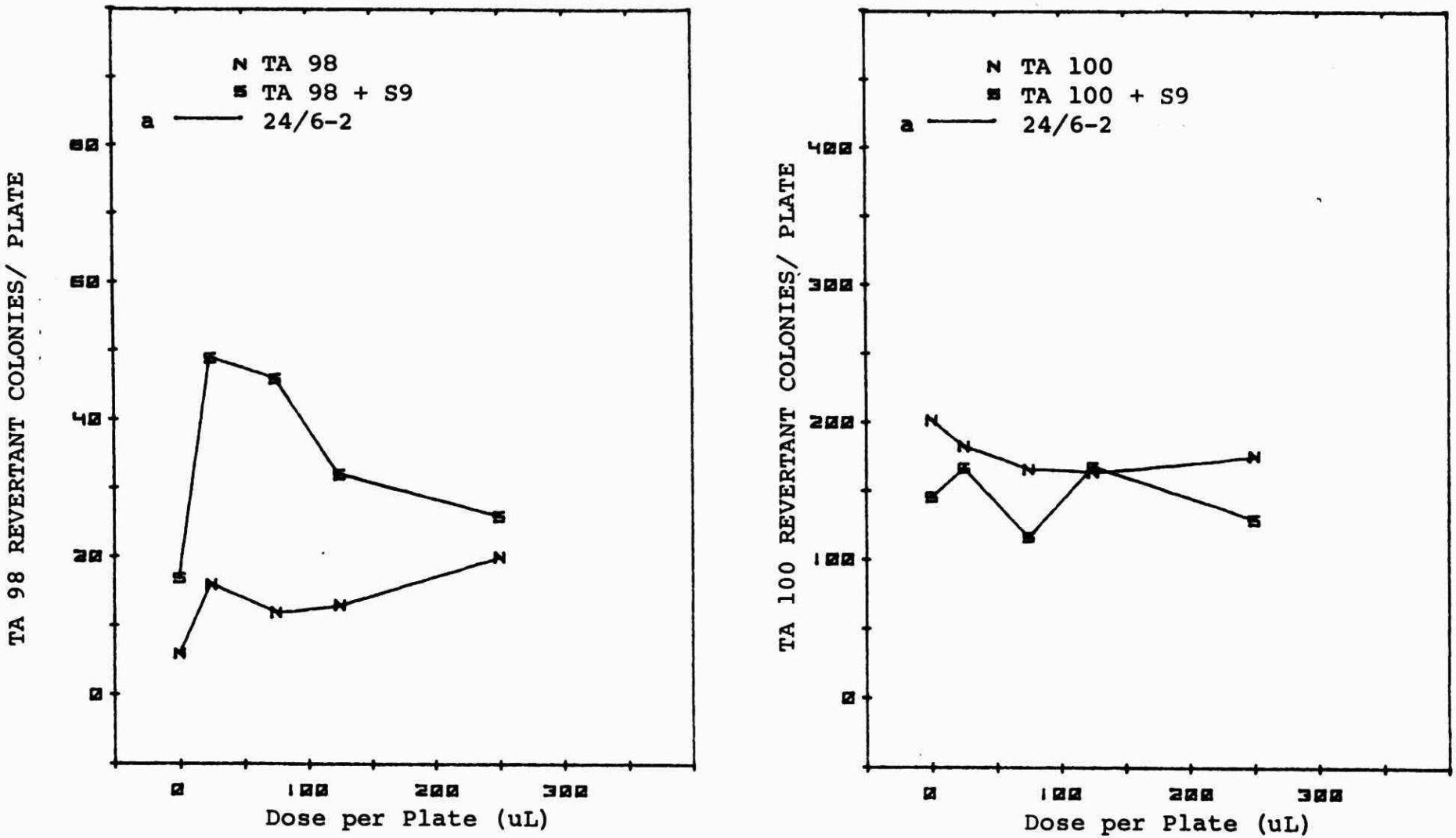




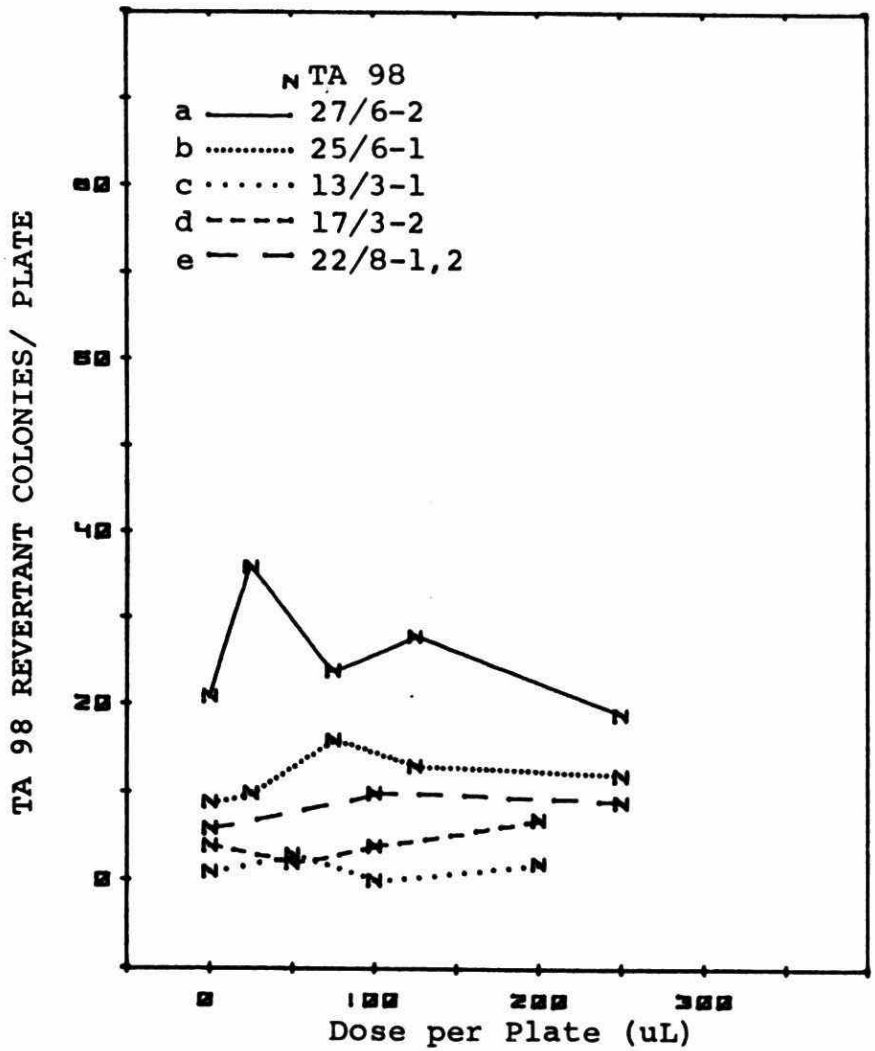
Appendix II A. Bacterial mutagenic response to the Township Ditch Out concentrate, Polysar Limited.
Positive controls: TA 98 + S9 and 2AF; a) 785 @2ug, b) 1145 @2ug
TA 100 and MNNG; a) 2767 @2ug, b) 2048 @2ug

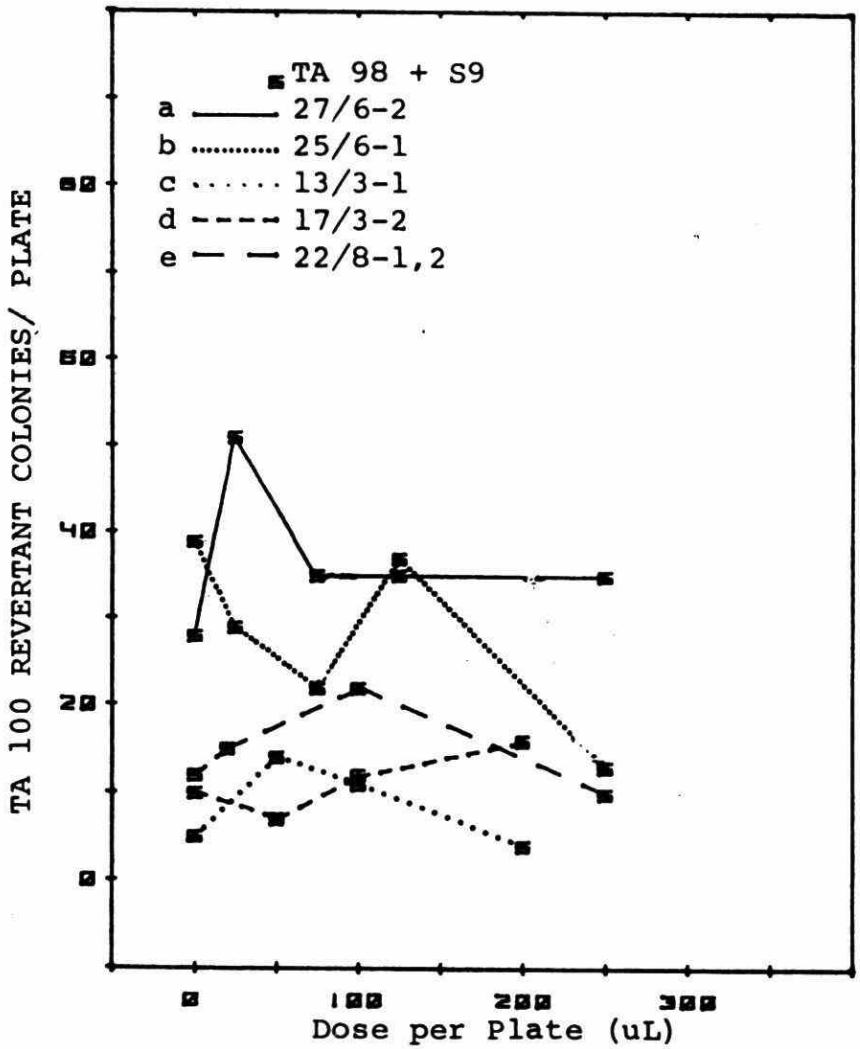


Appendix II B. Bacterial mutagenic response to the 54" Sewer effluent concentrate, Polysar Limited.
Positive controls: TA 98 + S9 and 2AF; a) 785 @2ug, b) 271 @4ug, c) 202 @4ug
TA 100 and MNNG; a) 2767 @2ug, b) 384 @2ug, c) 1114 @2ug

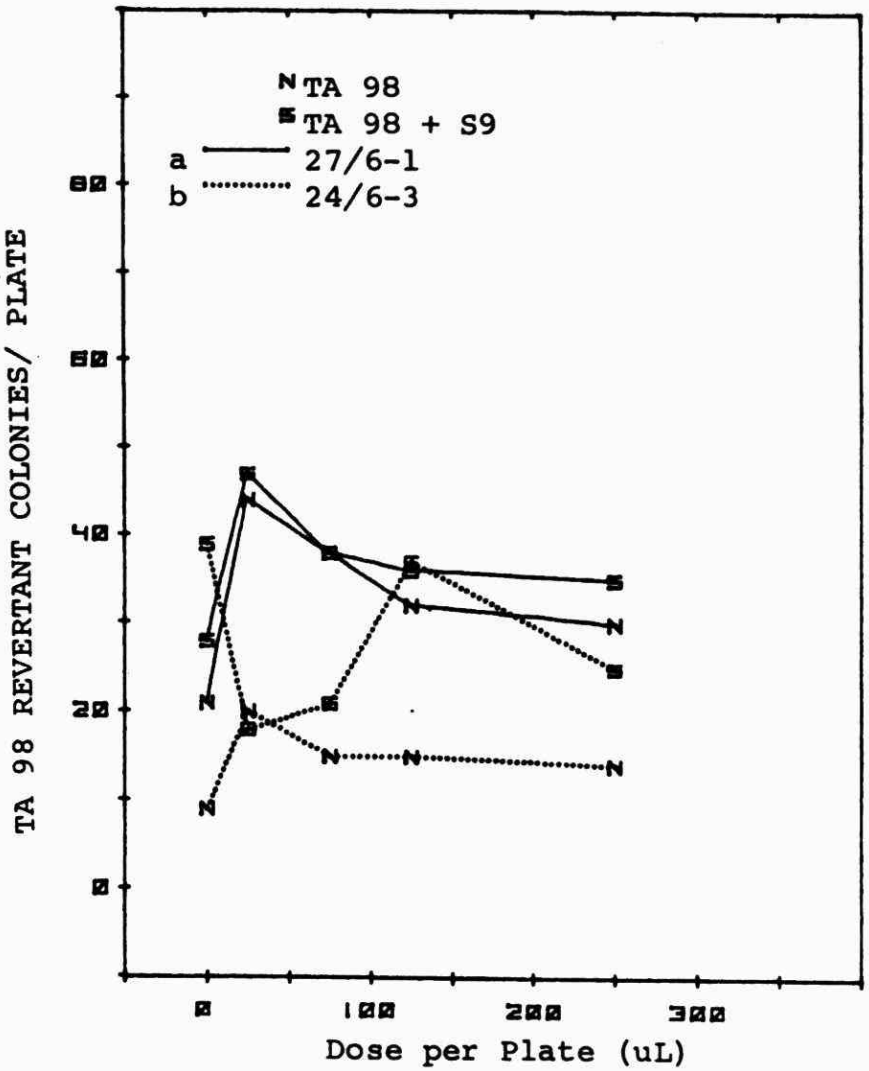


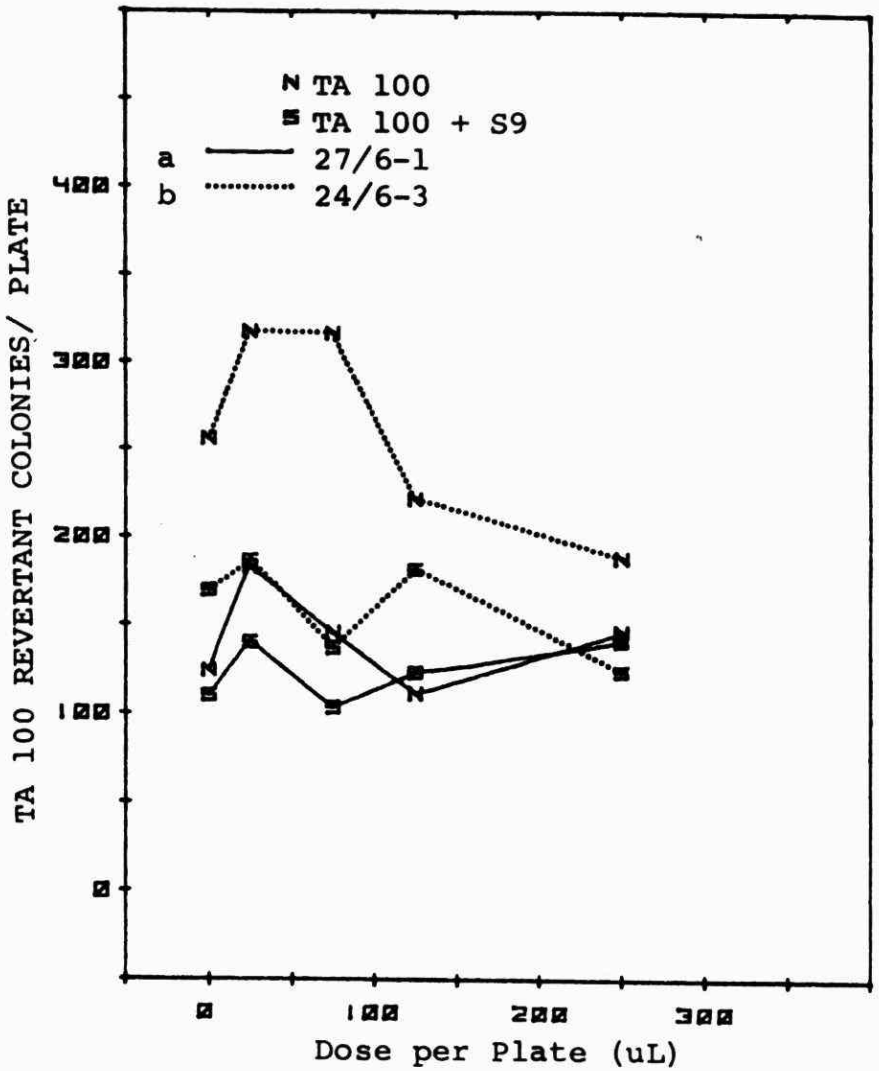
Appendix II C. Bacterial mutagenic response to the 66" Sewer effluent concentrate of Polysar Limited.
Positive controls: TA 98 + S9 and 2AF; a) 790 @2ug
TA 100 and MNNG; b) 1495 @2ug



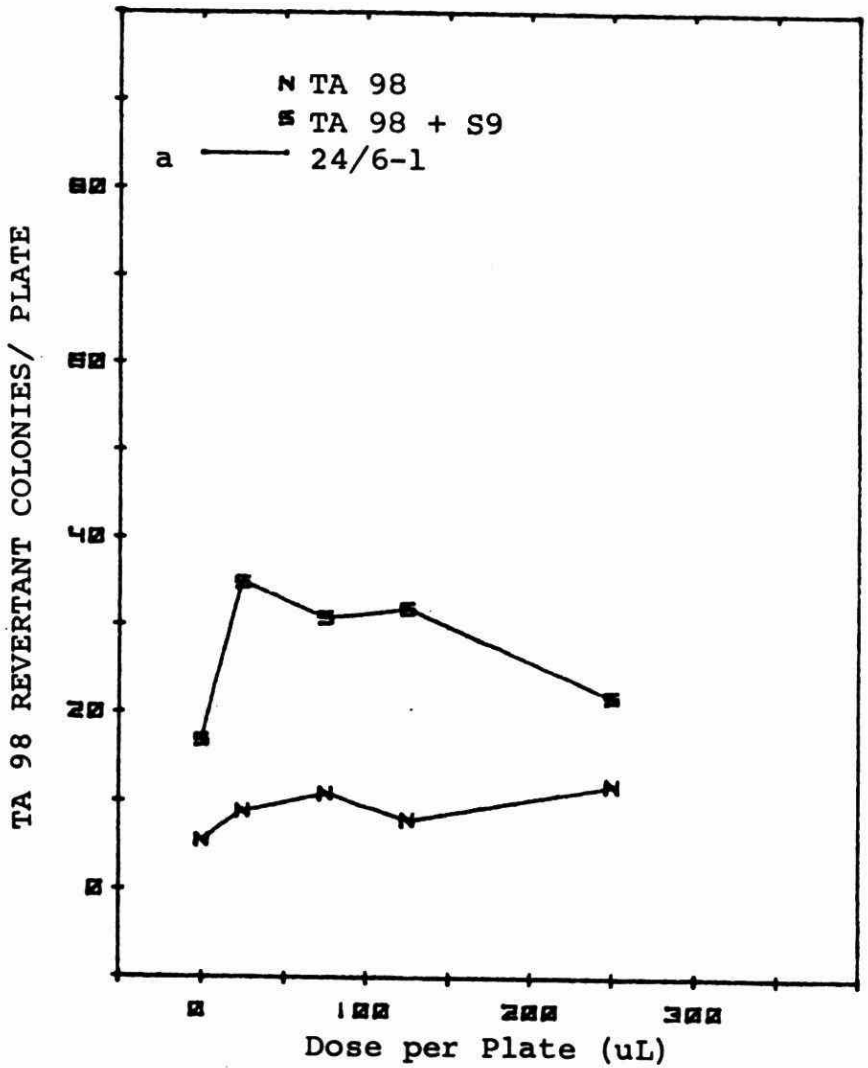


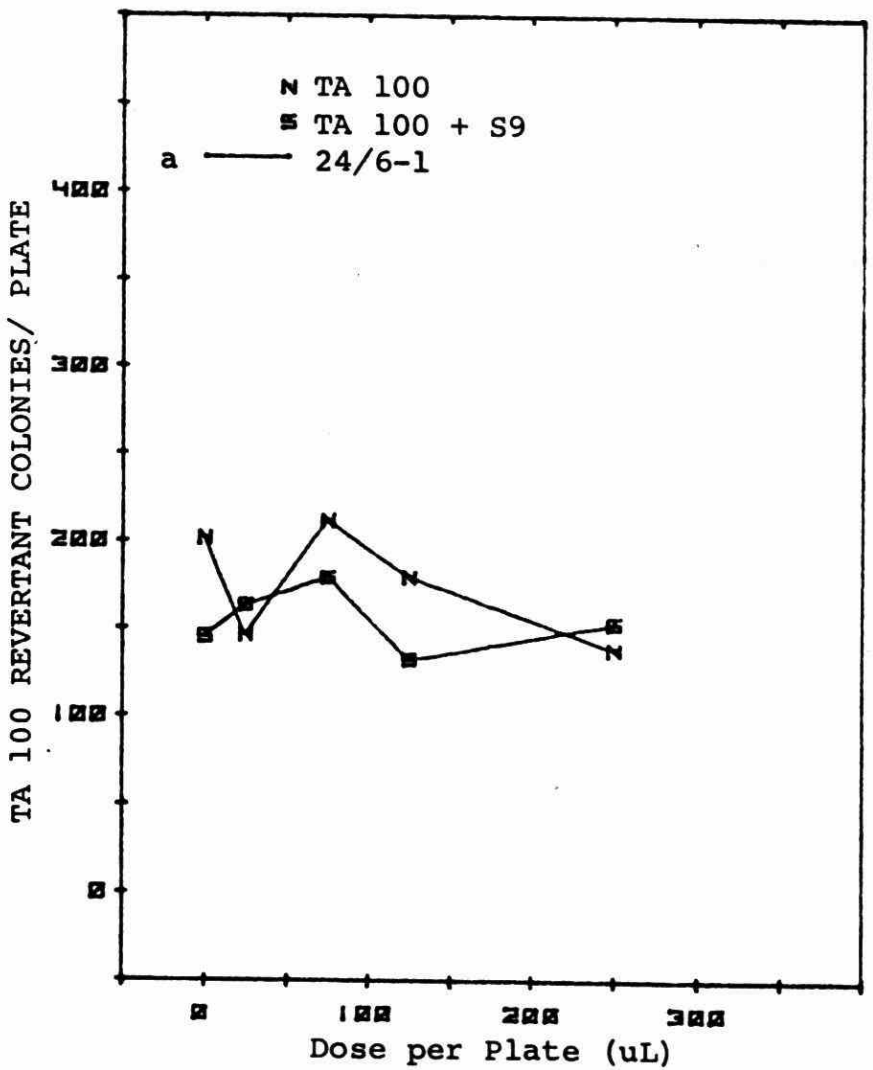
Appendis II D. Bacterial mutagenic response to the Stereo API effluent concentrate of Polysar Limited.
Positive controls: TA 98 + S9 and 2AF; a) 388 @2ug, b) 390 @2ug, c) 271 @4ug, d) 202 @4ug, e) 3833 @25ug



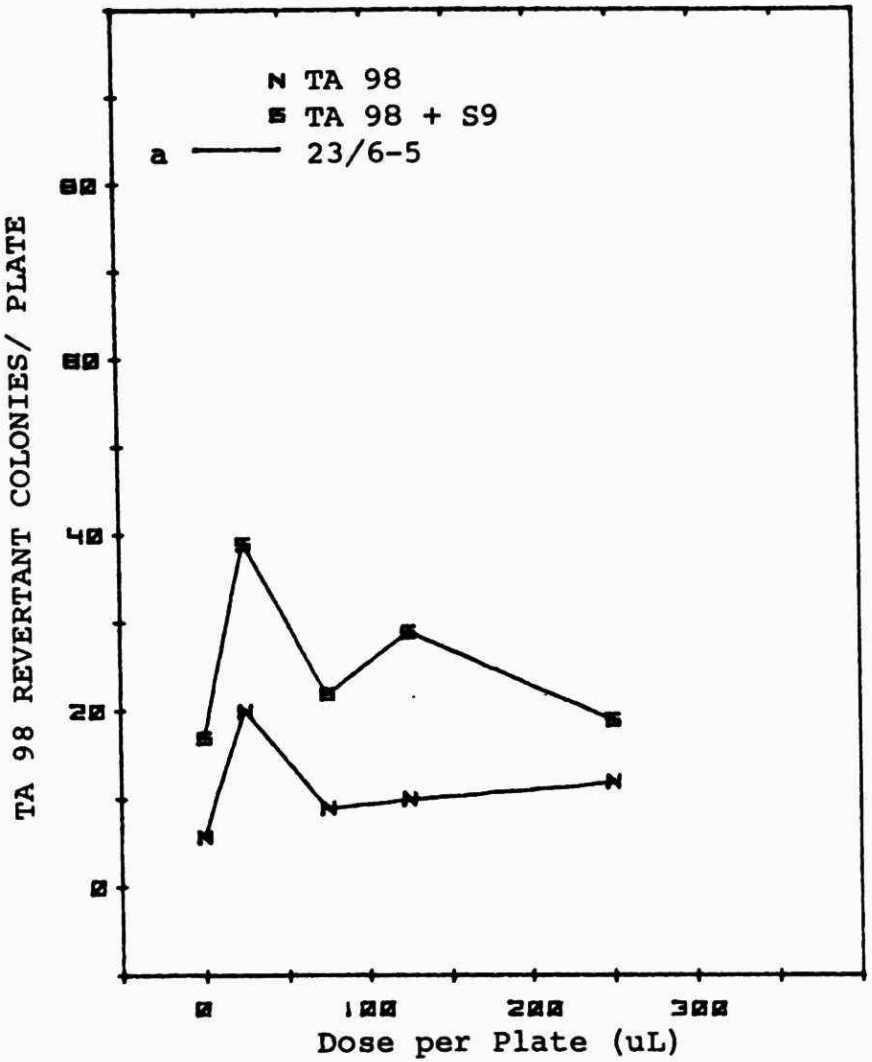


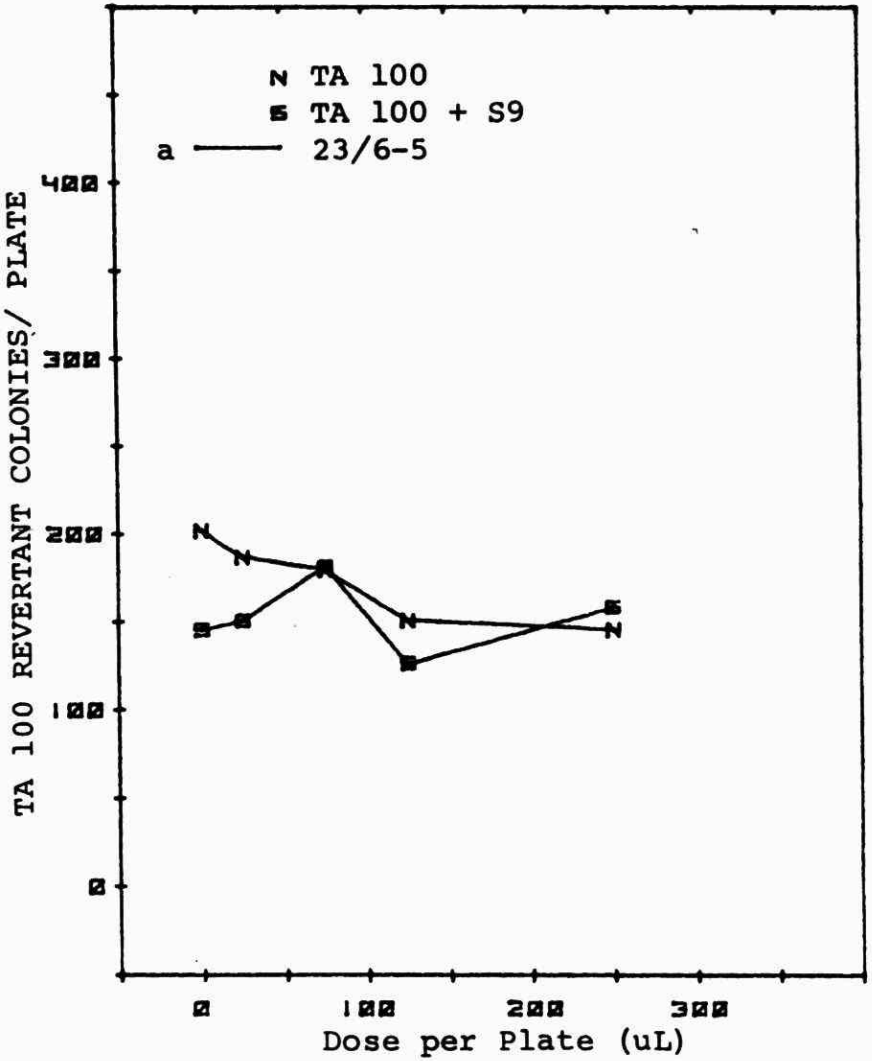
Appendix II E. Bacterial mutagenic response to the 72" Sewer effluent concentrate of Polysar Limited.
Positive controls: TA 98 + S9 and 2AF; a) 388 @2ug, b) 390 @2ug
TA 100 and MNNG; a) 4230 @2ug, b) 720 @2ug



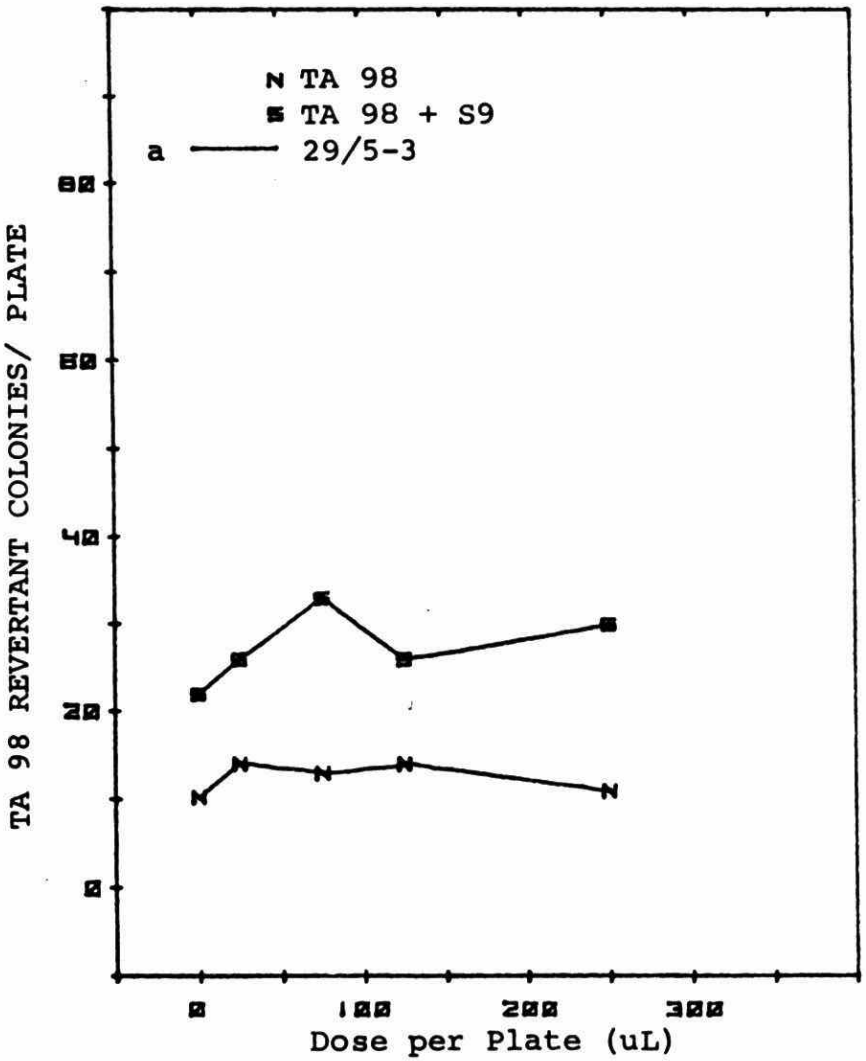


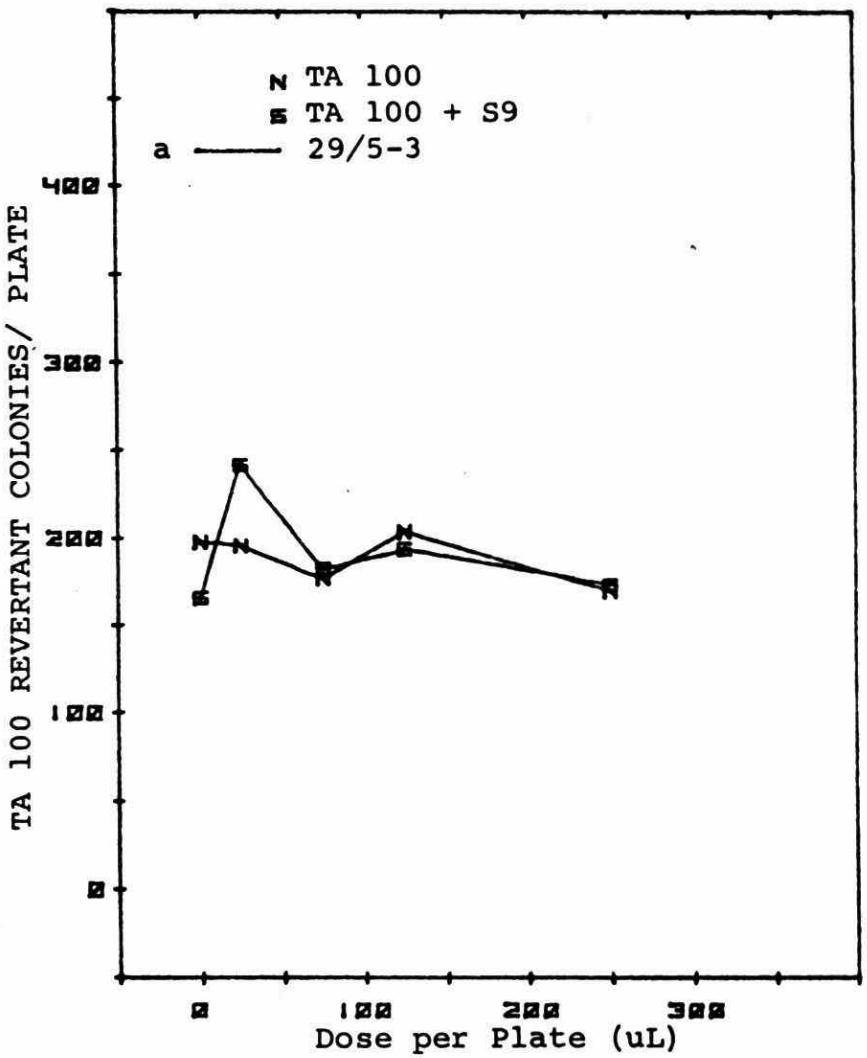
Appendix II F. Bacterial mutagenic response to the Township Ditch influent concentrate of Polysar Limited.
Positive controls: TA 98 + S9 and 2AF; a) 790 @2ug
TA 100 and MNNG; a) 1495 @2ug



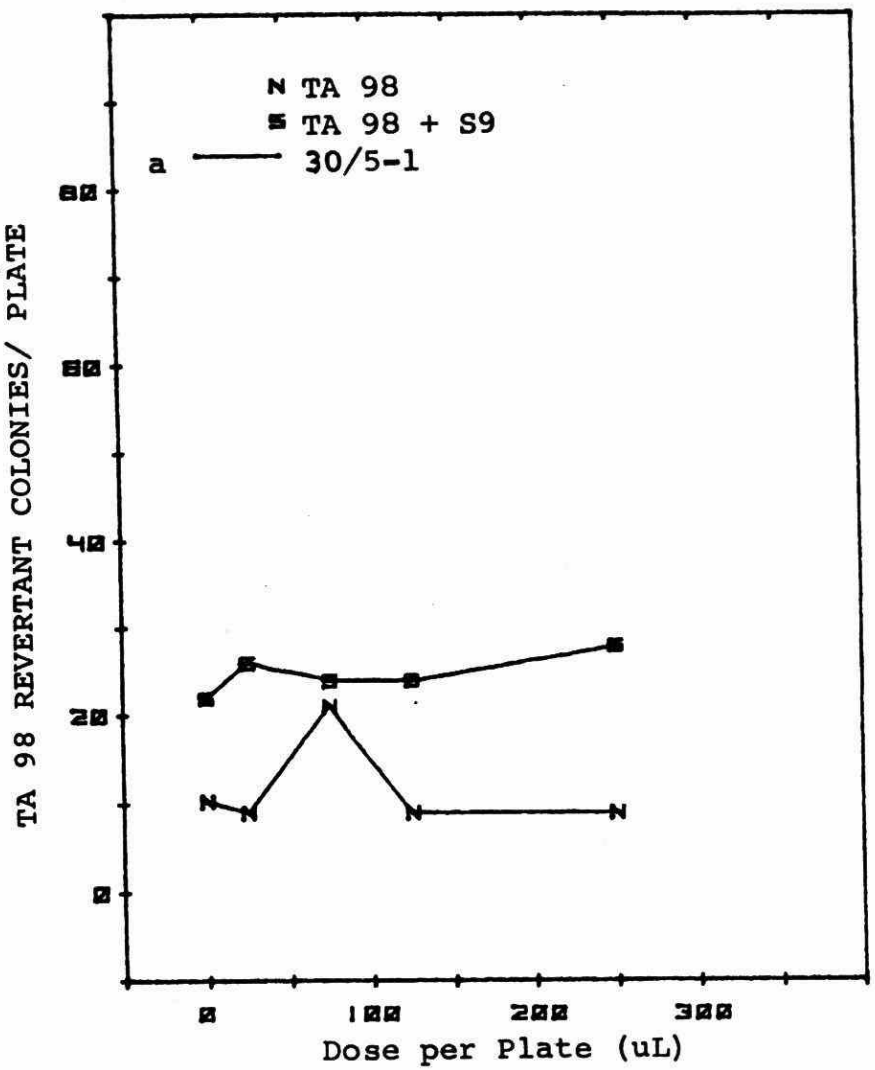


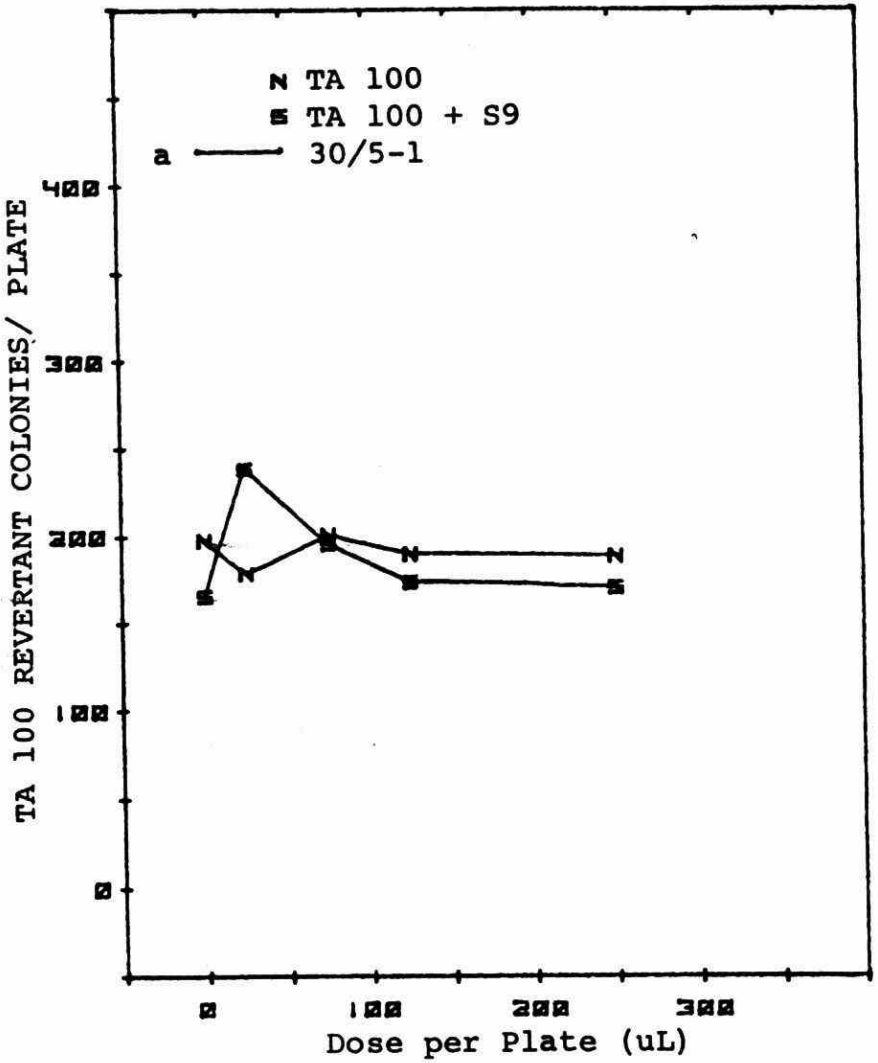
Appendix II G. Bacterial mutagenic response to the Service Water concentrate of Polysar Limited.
Positive controls: TA 98 + S9 and 2AF; a) 790 @2ug
TA 100 and MNNG; a) 1495 @2ug



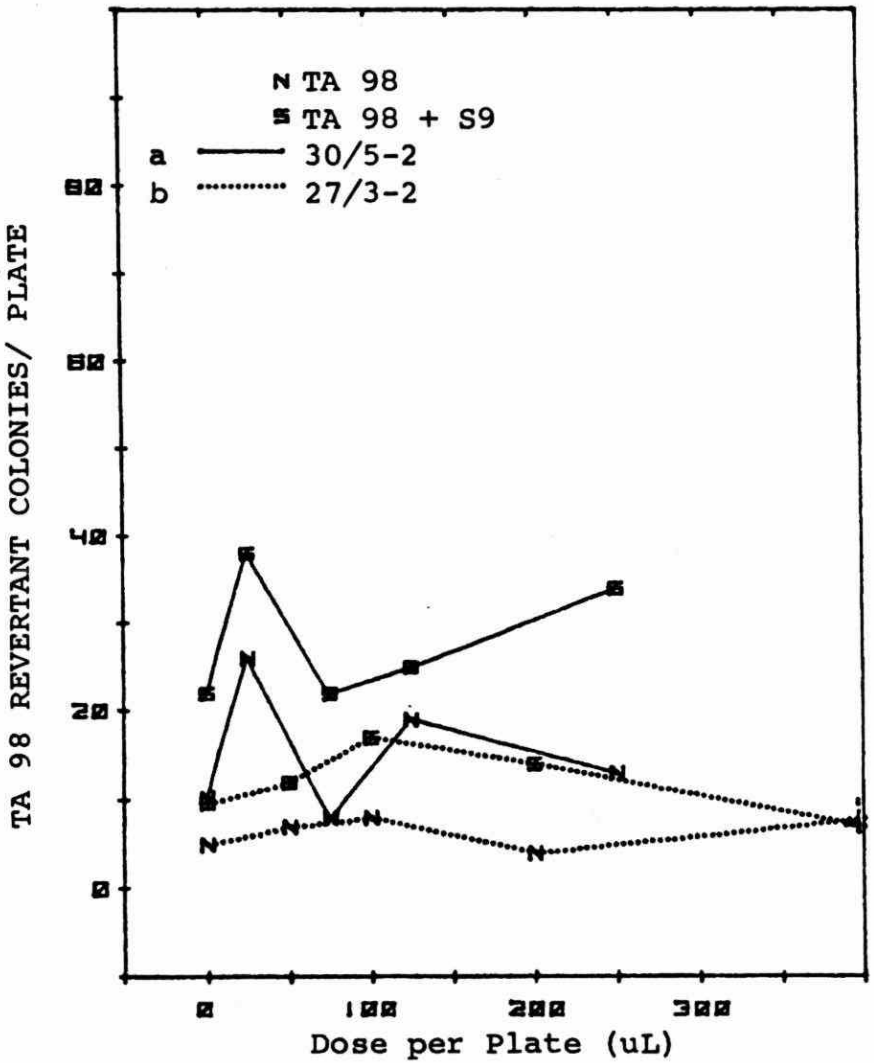


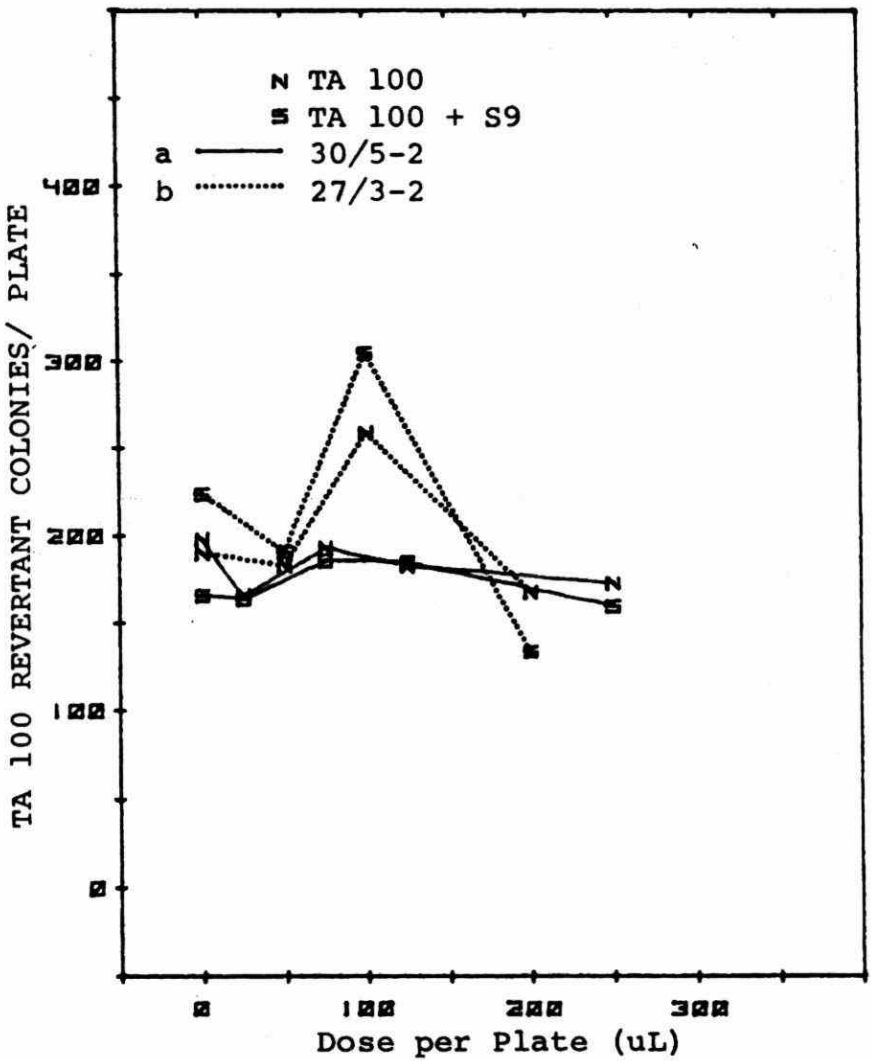
Appendix III A. Bacterial mutagenic response to the 42" Sewer effluent concentrate of Dow Chemical of Canada Limited.
Positive controls: TA 98 + S9 and 2AF; a) 1555 @2ug
TA 100 and MNNG; a) 915 @2ug



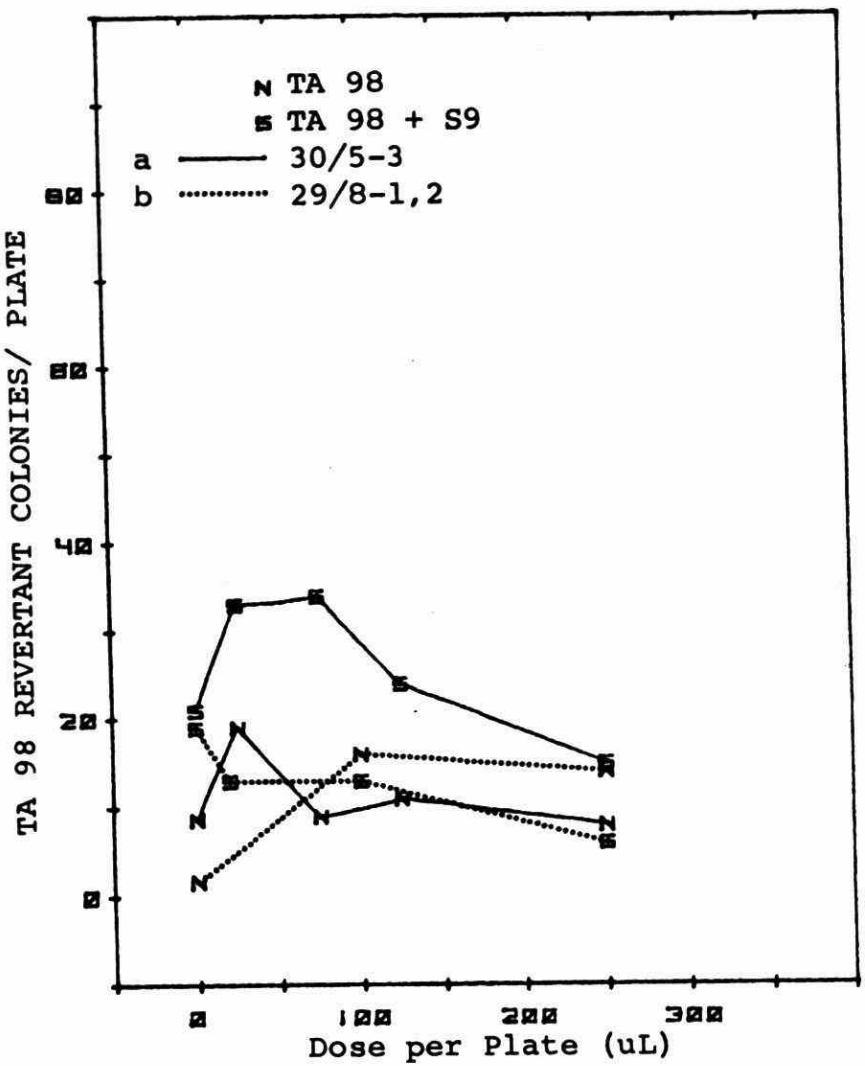


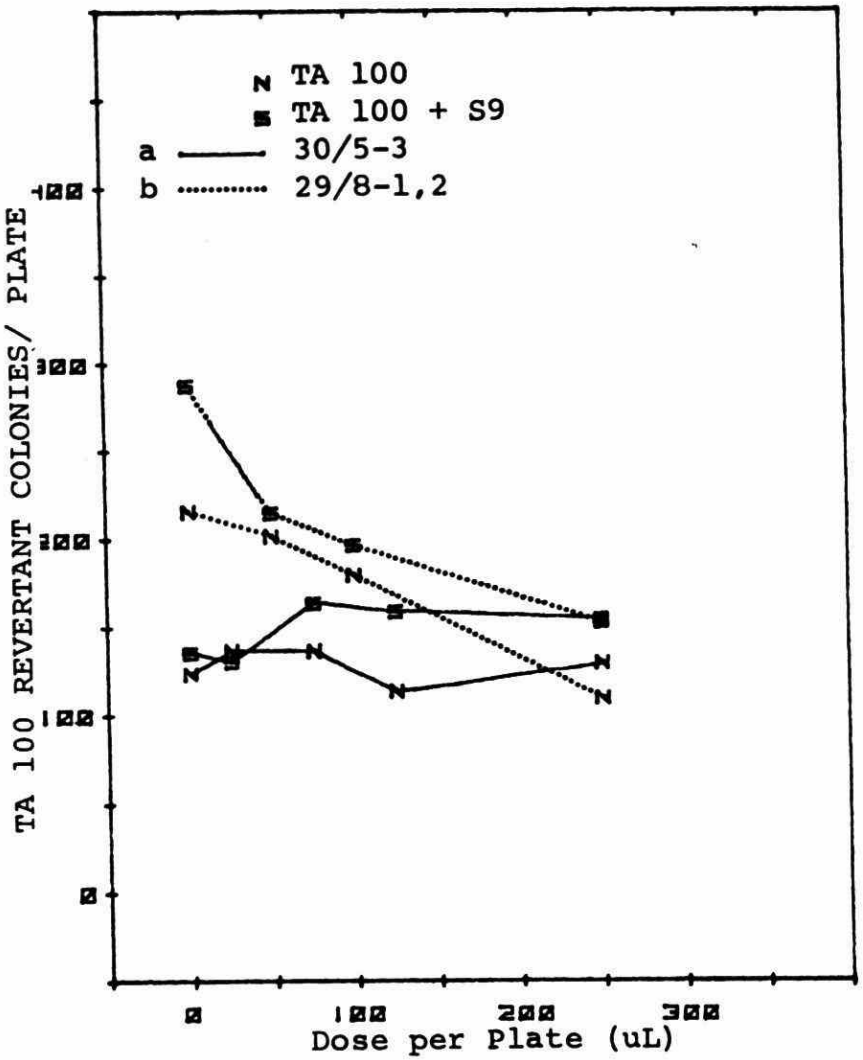
Appendix III B. Bacterial mutagenic response to the 48" Sewer effluent concentrate of Dow Chemical of Canada Limited.
Positive controls: TA 98 + S9 and 2AF; a) 1555 @2ug
TA 100 and MNNG; a) 915 @2ug



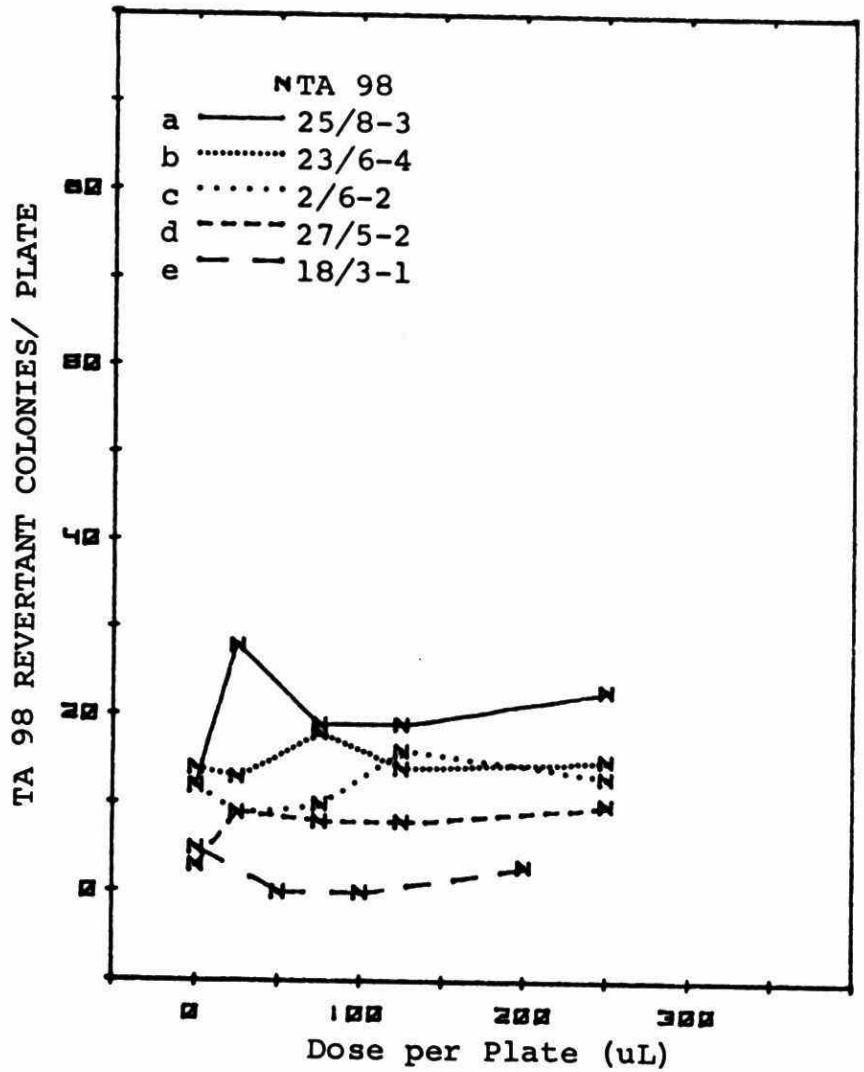


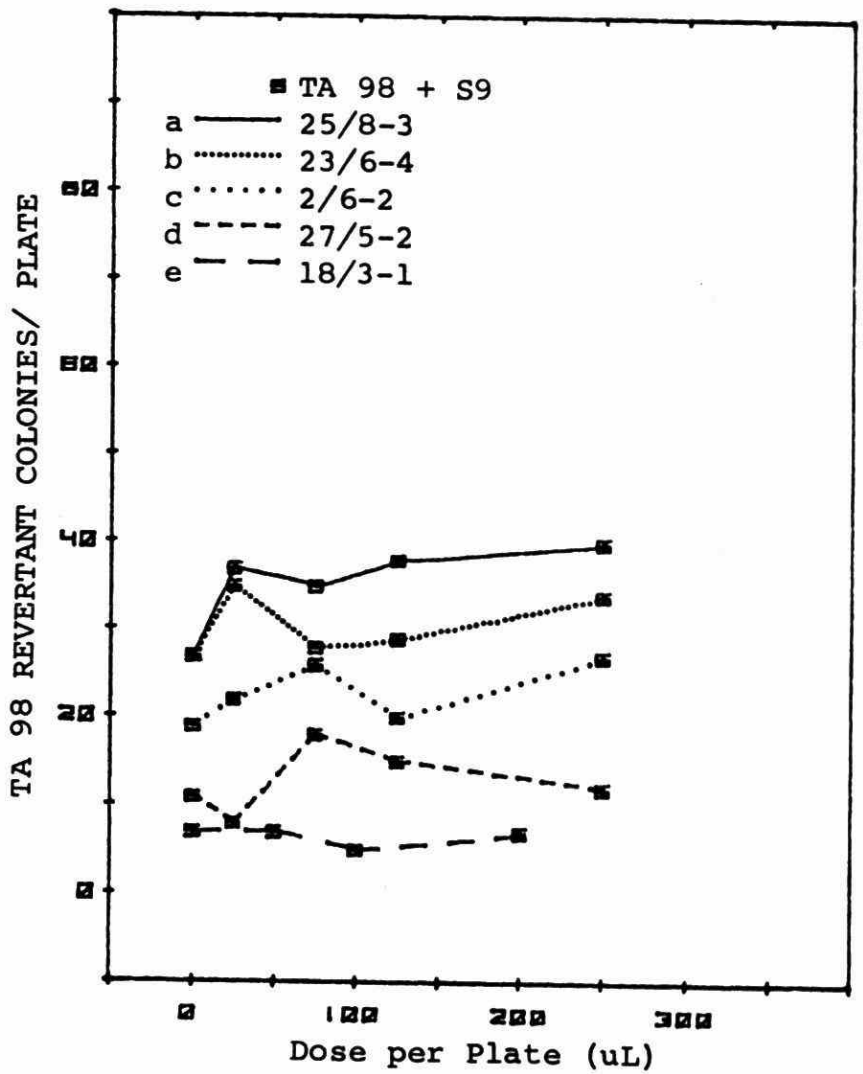
Appendix III C. Bacterial mutagenic response to the Acid Tile effluent concentrate of Dow Chemical of Canada Limited.
Positive controls: TA 98 + S9 and 2AF; a) 1555 @2ug, b) 2363 @4ug
TA 100 and MNNG; a) 630 @2ug, b) 7500 @20ug



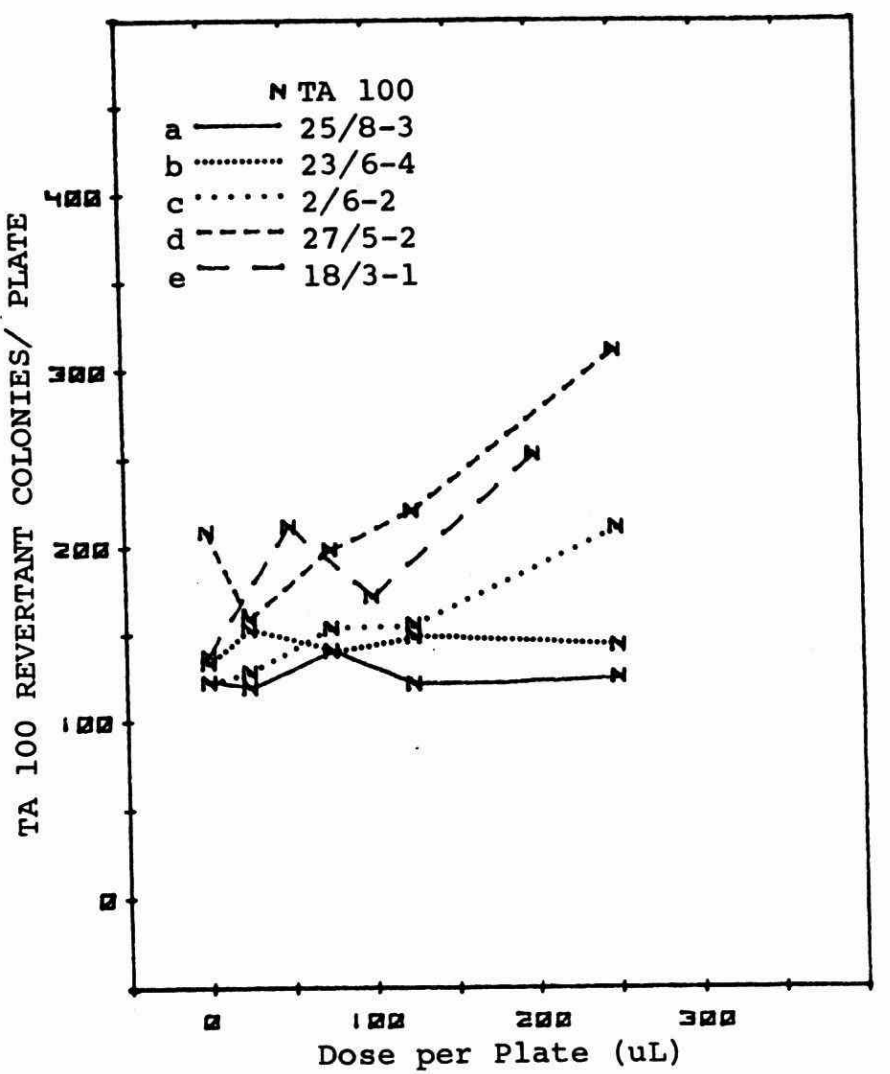


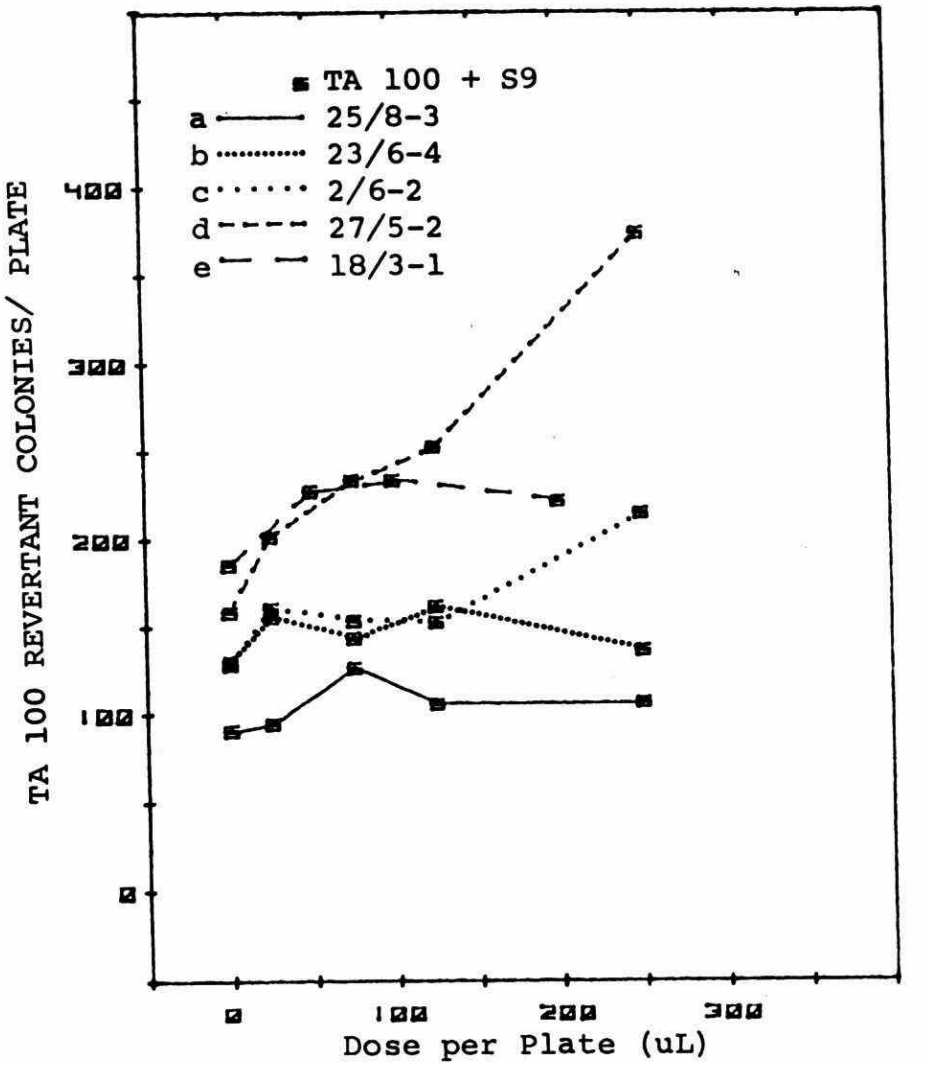
Appendix III D. Bacterial mutagenic response to the 54" Sluice effluent concentrate of Dow Chemical of Canada Limited.
Positive controls: TA 98 + S9 and 2AF; a) 1260 @2ug, b) 2433 @2.5ug
TA 100 and MNNG; a) 2490 @2ug, b) >10000 @10ug



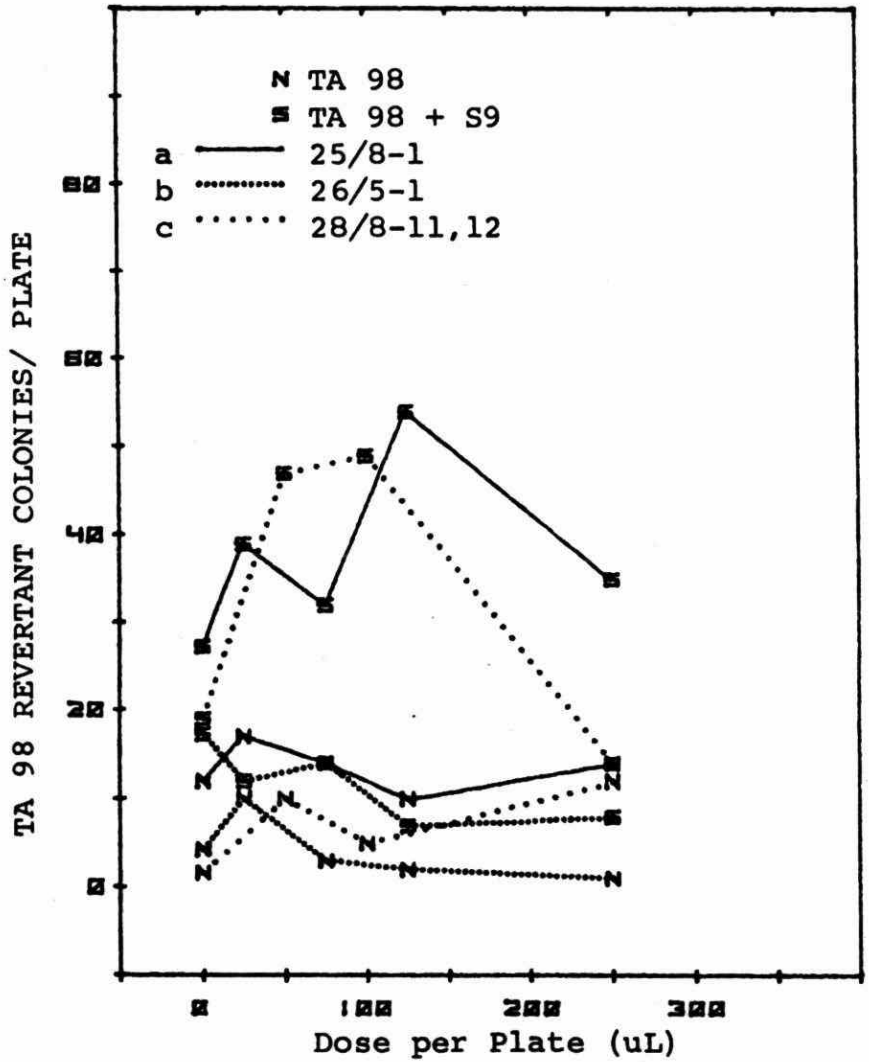


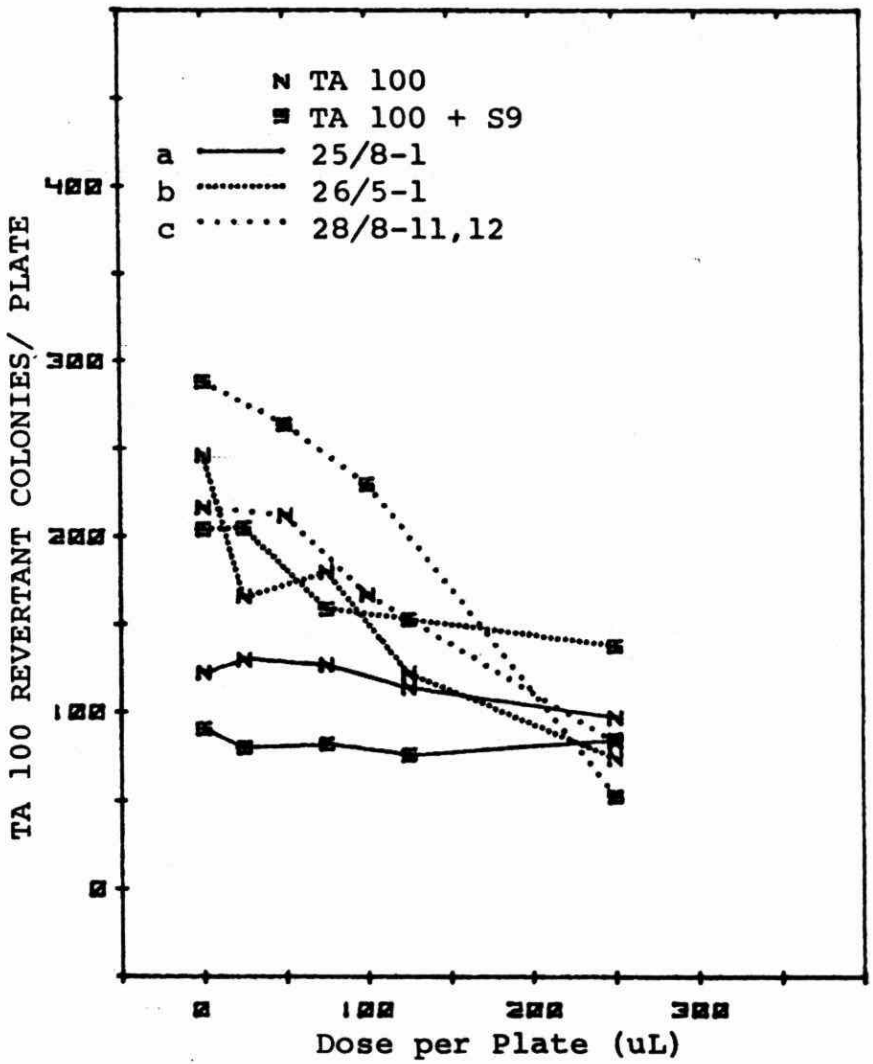
Appendix III E. Bacterial mutagenic response to the 2nd Street Sewer effluent concentrate of Dow Chemical of Canada Limited.
Positive controls: TA 98 + S9 and 2AF; a) 510 @2ug, b) 785 @2ug, c) 1360 @2ug
d) 598 @2ug, e) 1677 @4ug



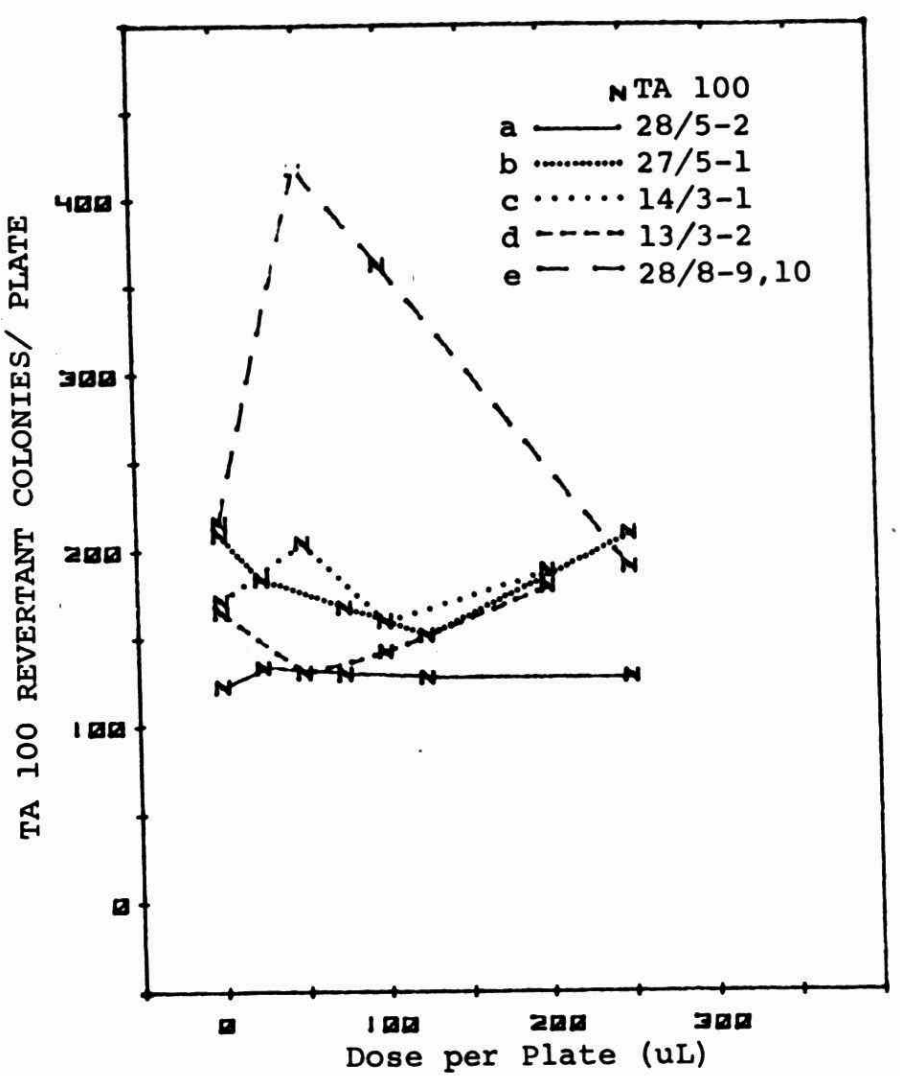


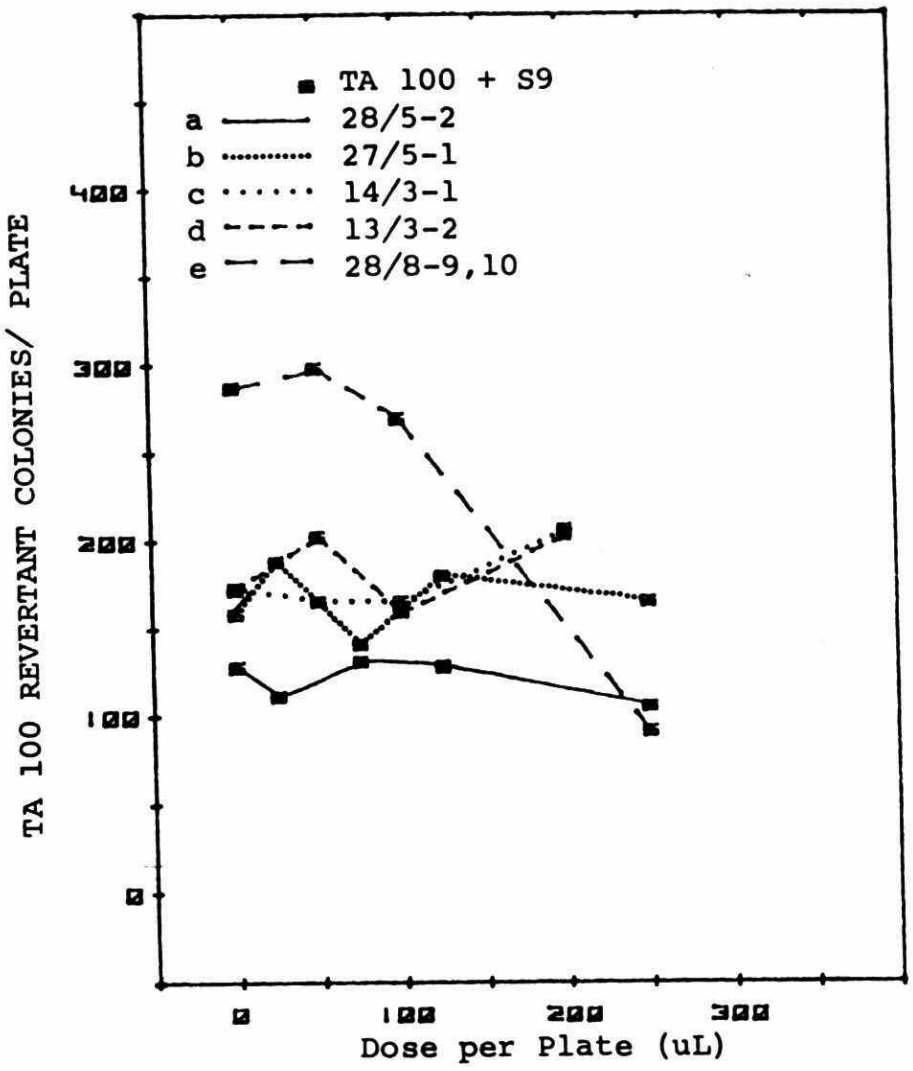
Appendix III E continued. Bacterial mutagenic response to the 2nd Street Sewer effluent concentrate of Dow Chemical of Canada Limited
Positive controls: TA 100 and MNNG; a) 3080 @2ug, b) 2767 @2ug, c) 2847 @2ug, d) 388 @2ug, e) 1014 @4ug



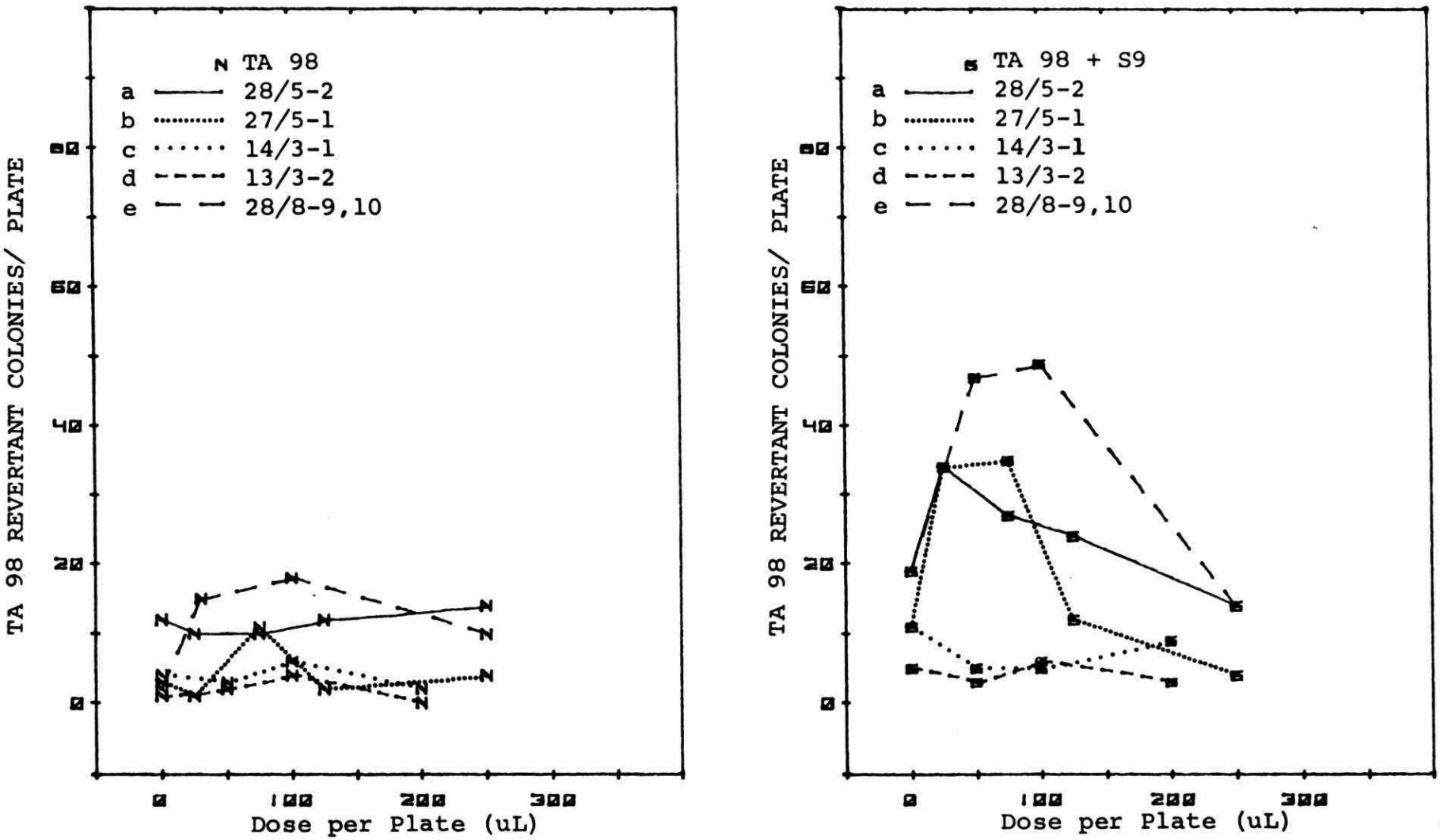


Appendix III F. Bacterial mutagenic response to the 3rd Street Sewer effluent concentrate of Dow Chemical of Canada Limited.
Positive controls: TA 98 + S9 and 2AF; a) 510 @2ug, b) 1165 @2ug, c) 2433 @2.5ug
TA 100 and MNNG; a) 3080 @2ug, b) 1189 @2ug, c) >10000@10ug

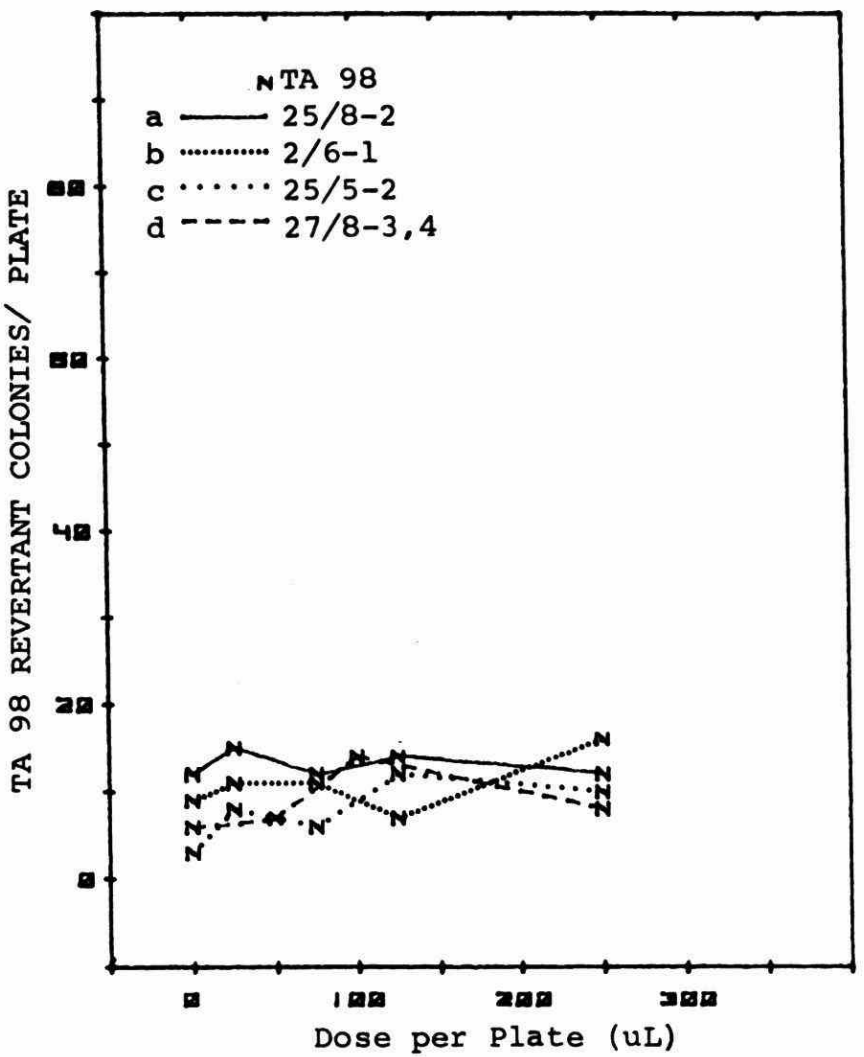


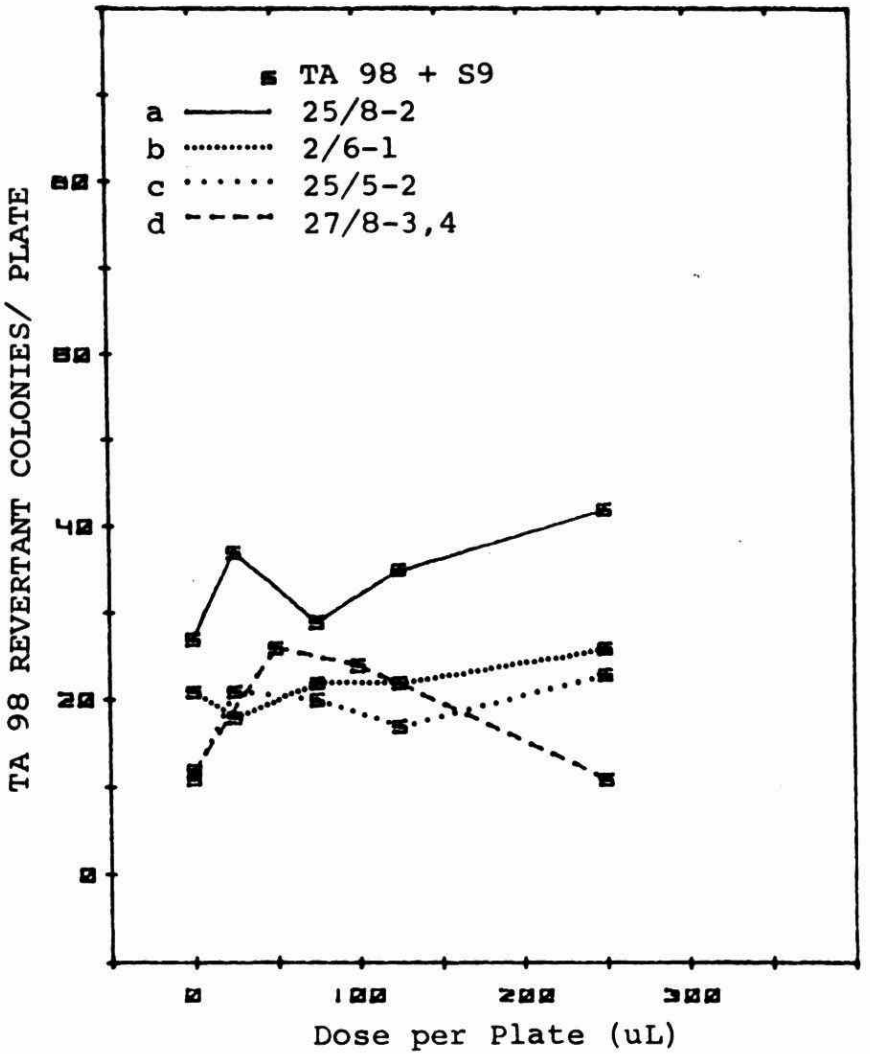


Appendix III G continued. Bacterial mutagenic response to concentrate of samples from the Direct Oxidation of Ethylene Oxide (DOEO) effluent, Dow Chemical of Canada Limited. Positive controls: TA 100 and MNNG; a) 2880 @2ug, b) 388 @2ug, c) 1114 @4ug, d) 384 @2ug, e) >10000 @10ug

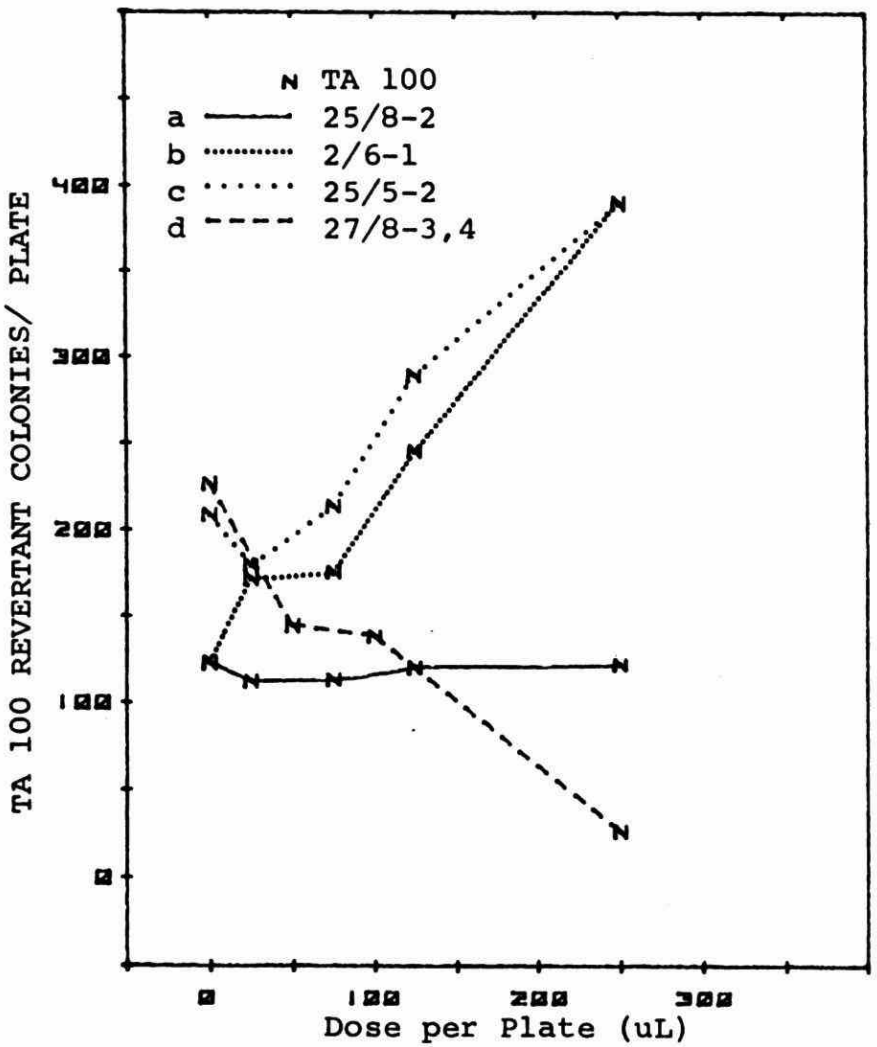


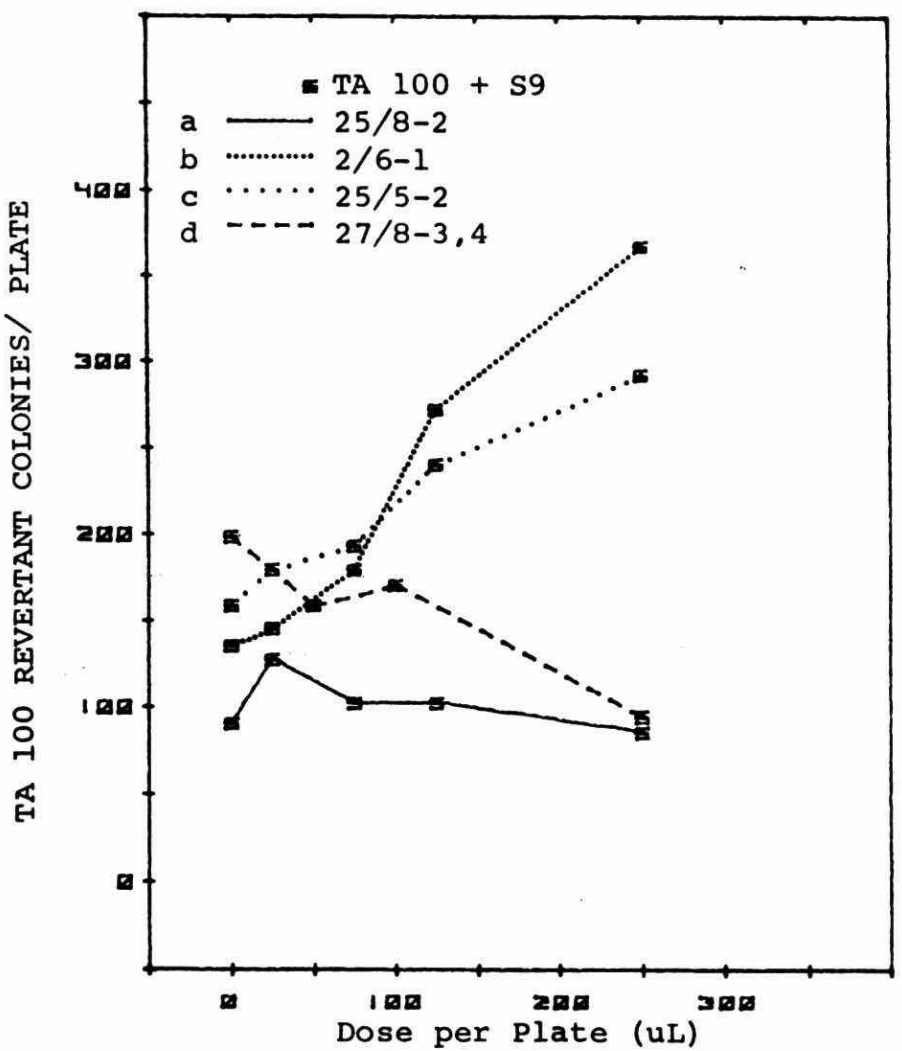
Appendix III G. Bacterial mutagenic response to concentrate of samples from the Direct Oxidation of Ethylene Oxide (DOEO) effluent, Dow Chemical of Canada Limited. Positive controls: TA 98 + S9 and 2AF; a) 1360 @2ug, b) 598 @2ug, c) 202 @4ug, d) 271 @4ug, e) 4100 @2.5ug



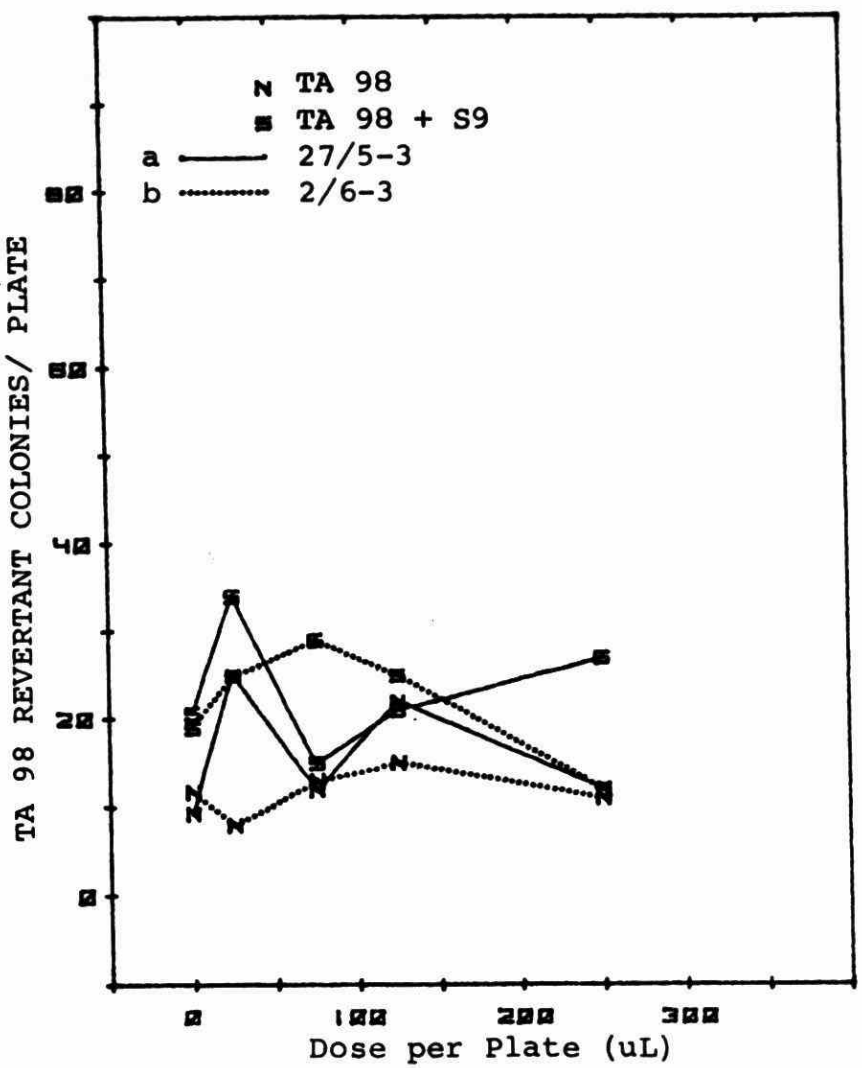


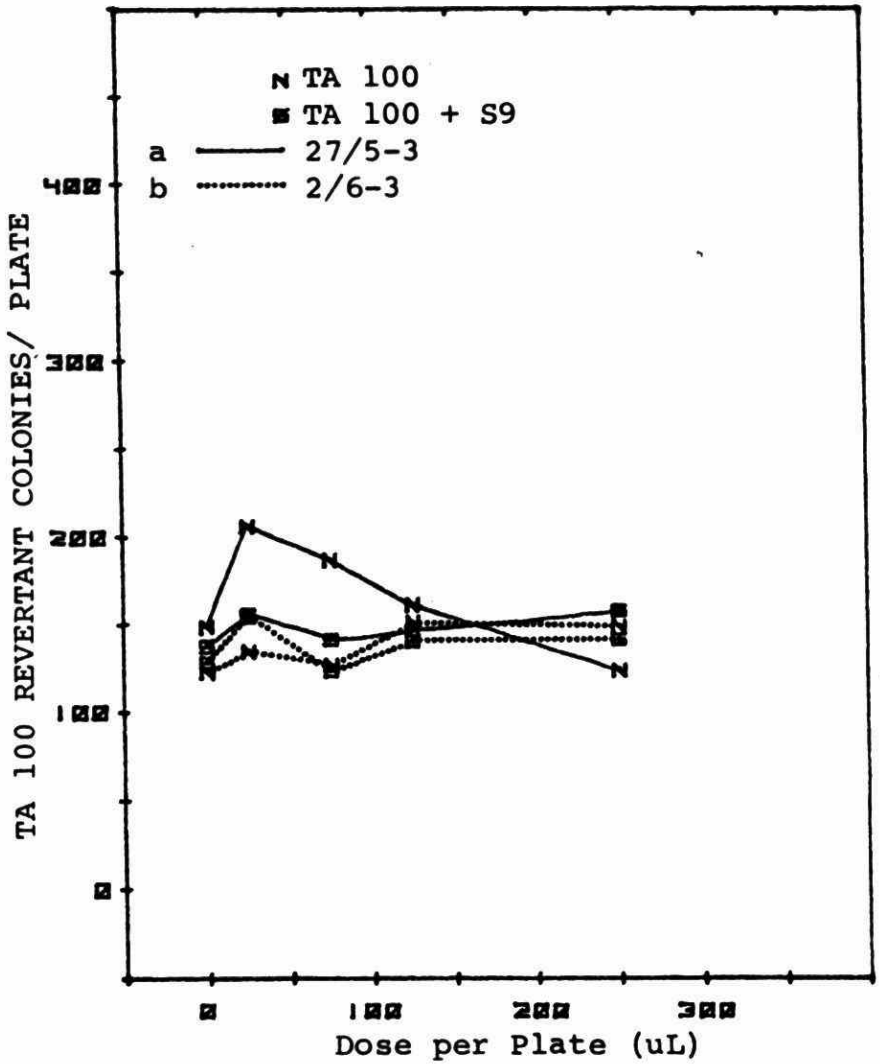
Appendix III H. Bacterial mutagenic response to the 4th Street Sewer effluent concentrate of Dow Chemical of Canada Limited.
Positive controls: TA 98 + S9 and 2AF; a) 510 @2ug, b) 1260 @2ug, c) 598 @2ug, d) 3700 @2.5ug



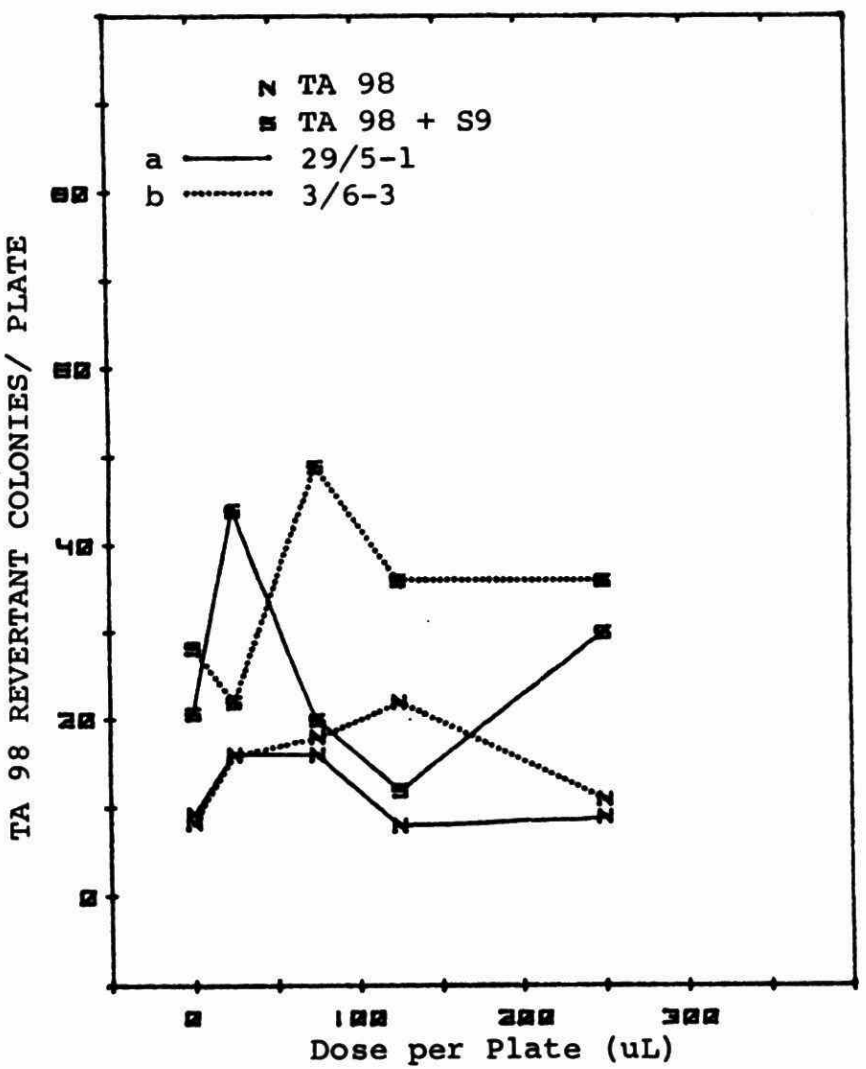


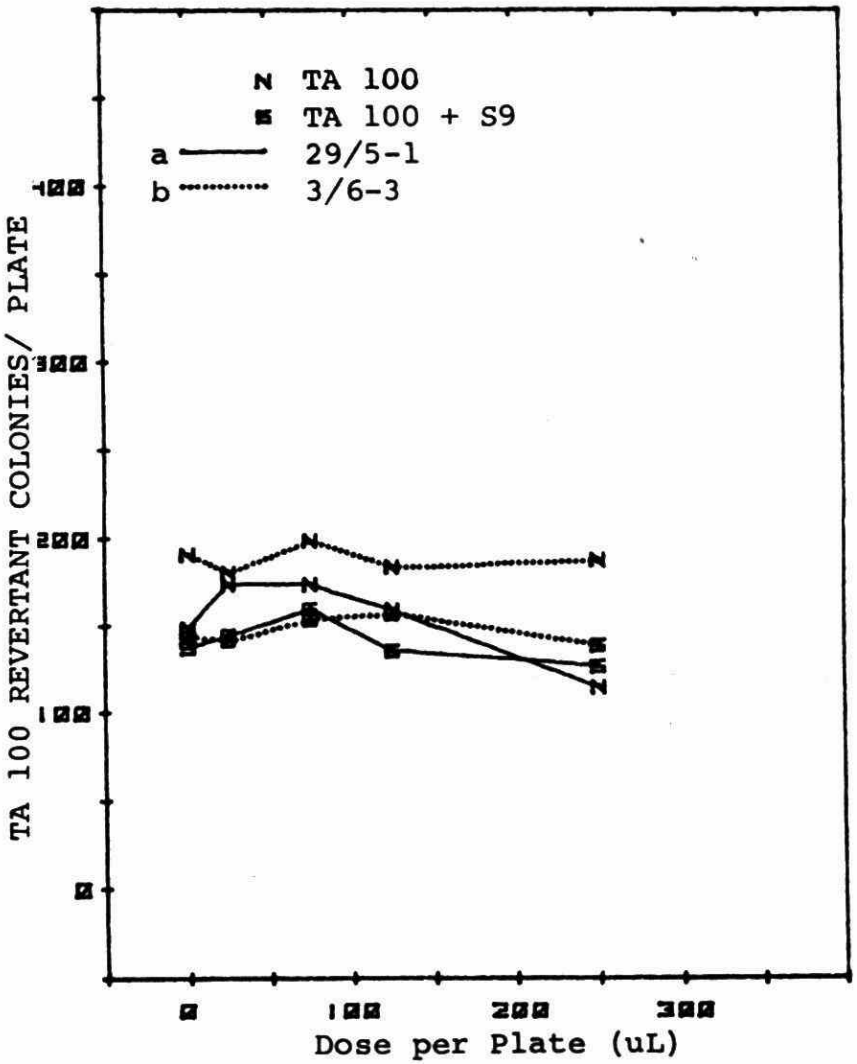
Appendix III H. continued Bacterial mutagenic response to the 4th Street Sewer effluent concentrate of Dow Chemical of Canada Limited.
Positive controls: TA 100 and MNNG; a) 3080 @2ug. b) 2490 @2ug, c) 388 @2ug, d) 5533 @10 ug



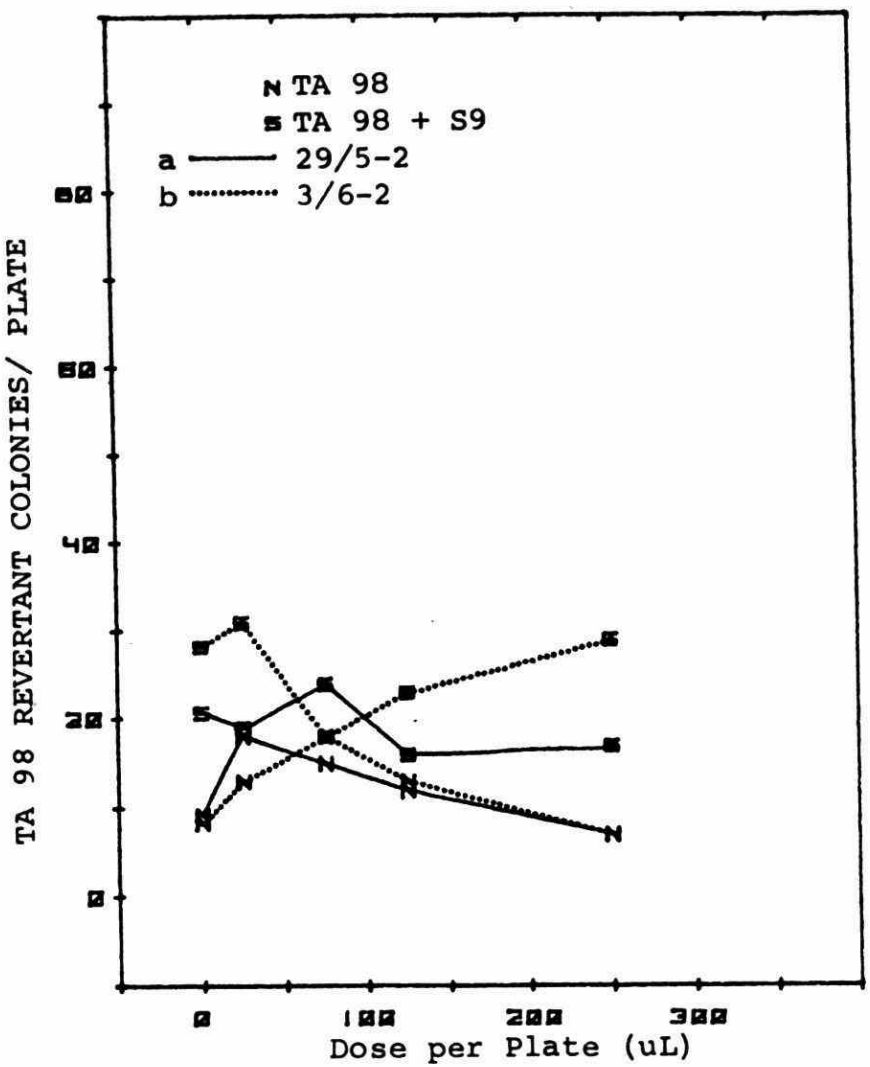


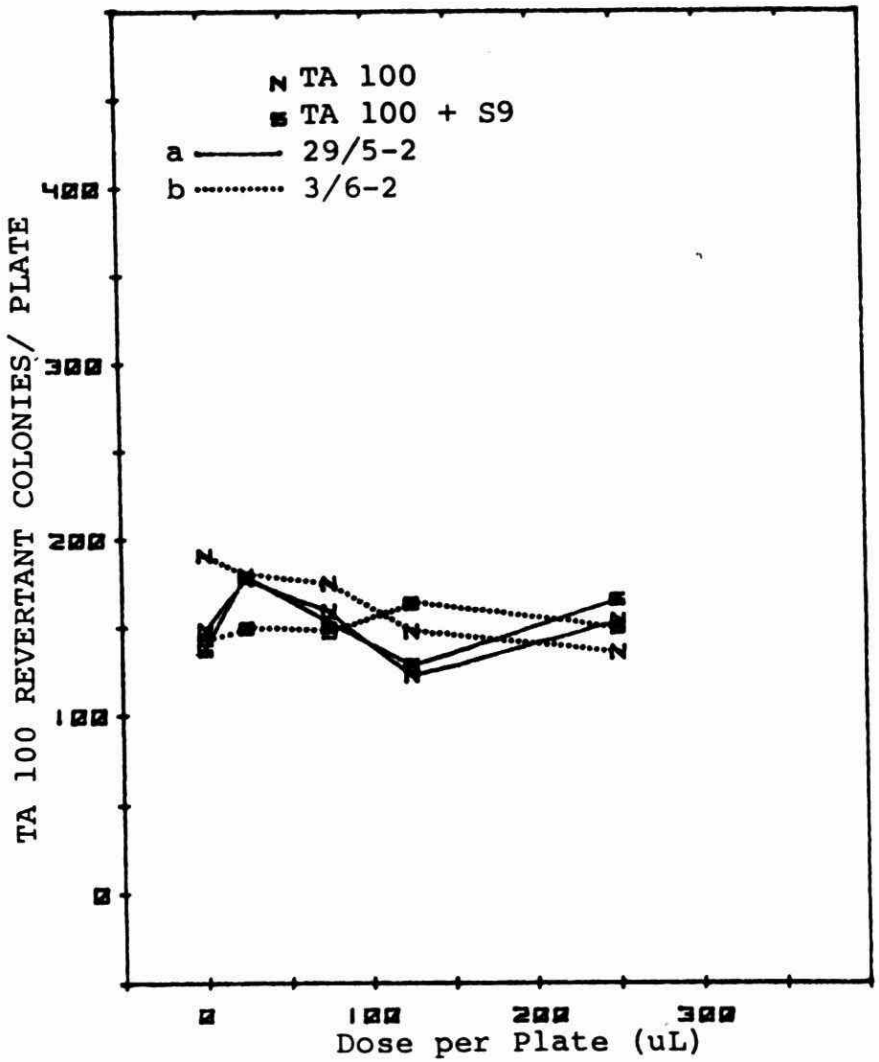
Appendix III I. Bacterial mutagenic response to the 4th Street Service Water concentrate of Dow Chemical of Canada Limited.
Positive controls: TA 98 + S9 and 2AF; a) 1270 @2ug, b) 1360 @2ug
TA 100 and MNNG; a) 369 @2ug, b) 4213 @2ug



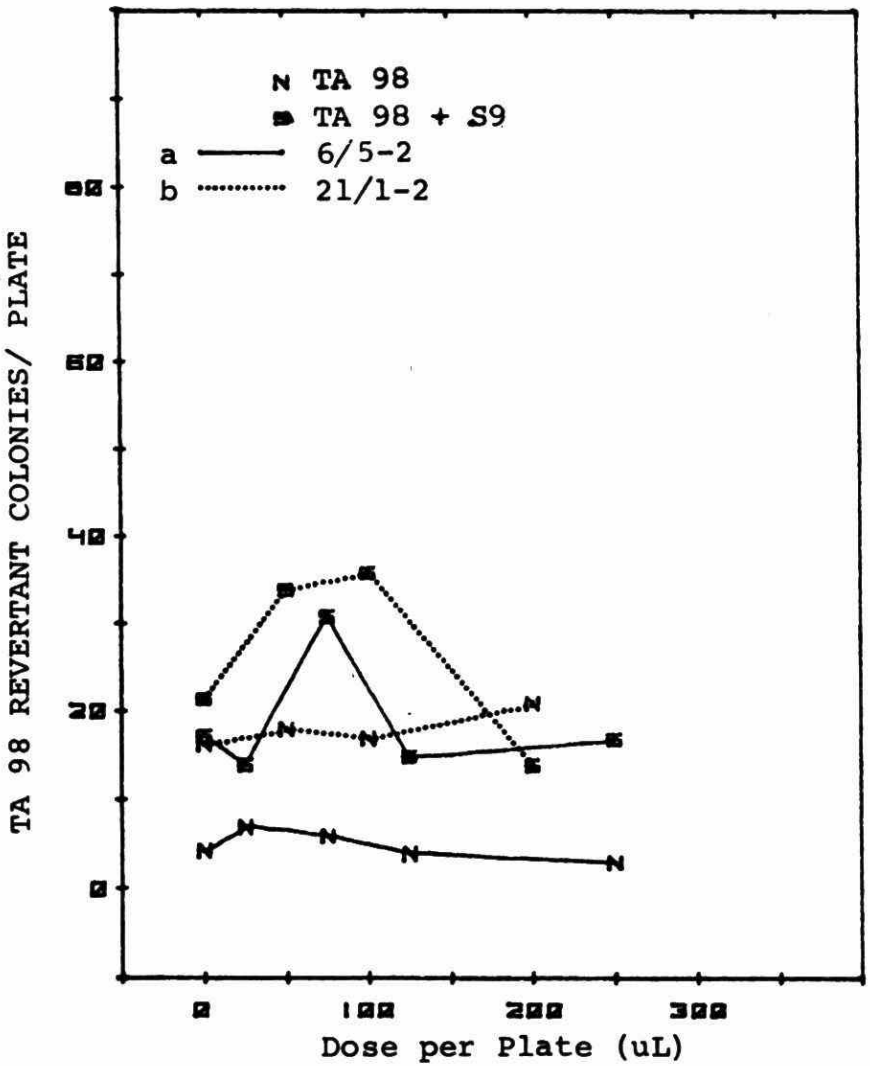


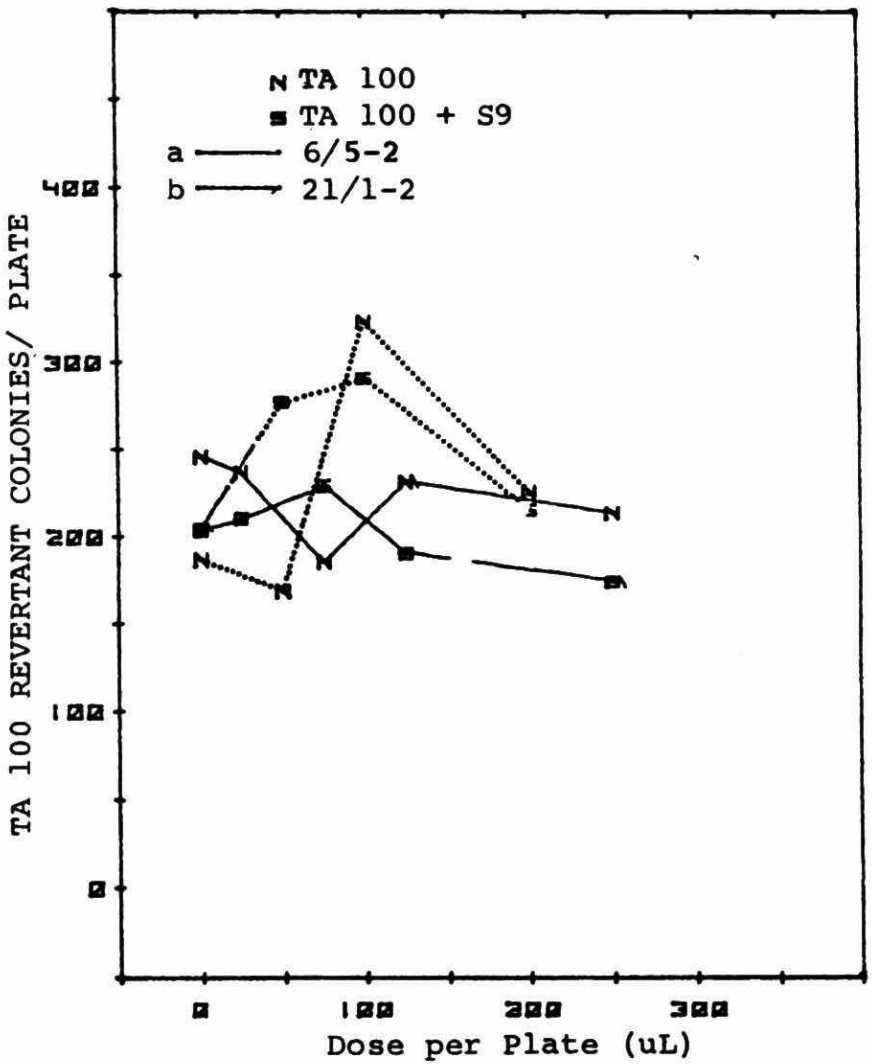
Appendix III J. Bacterial mutagenic response to the 3rd Street Service Water concentrate of Dow Chemical of Canada Limited.
Positive controls: TA 98 + S9 and 2AF; a) 1270 @2ug, b) 1220 @2ug
TA 100 and MNNG; a) 369 @2ug, b) 1335 @2ug



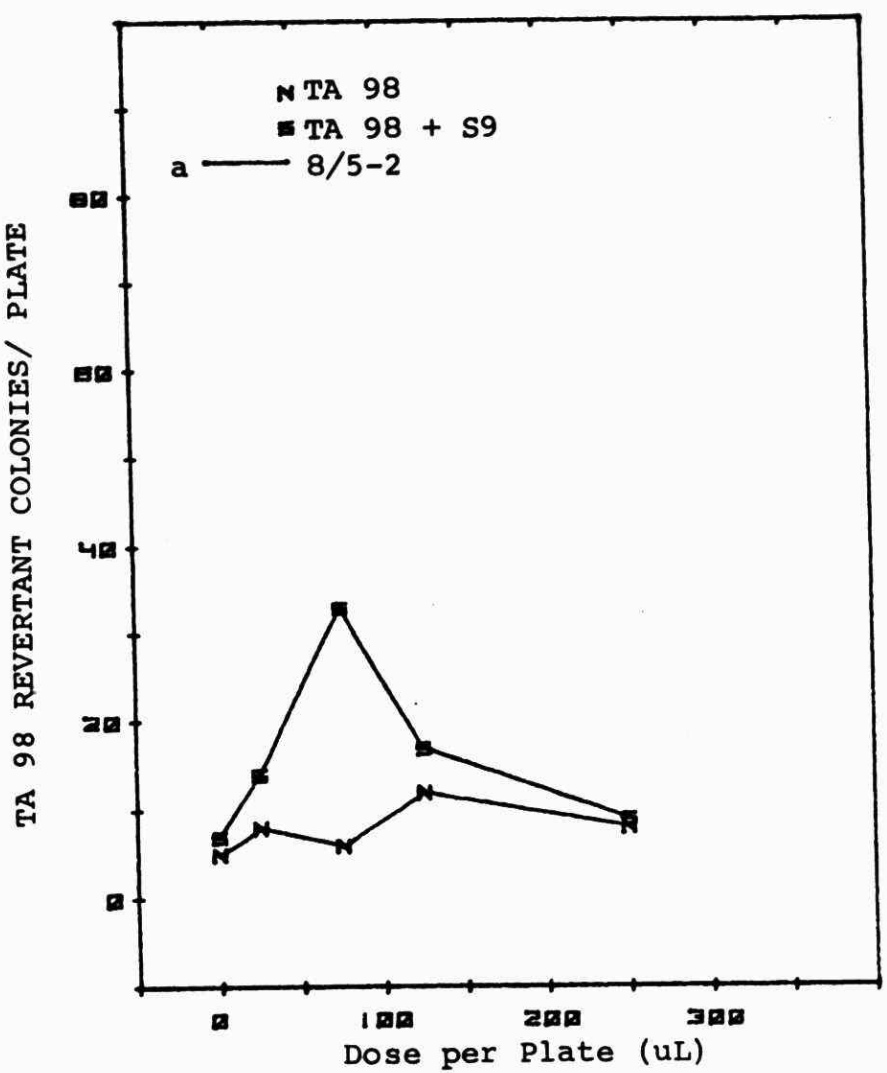


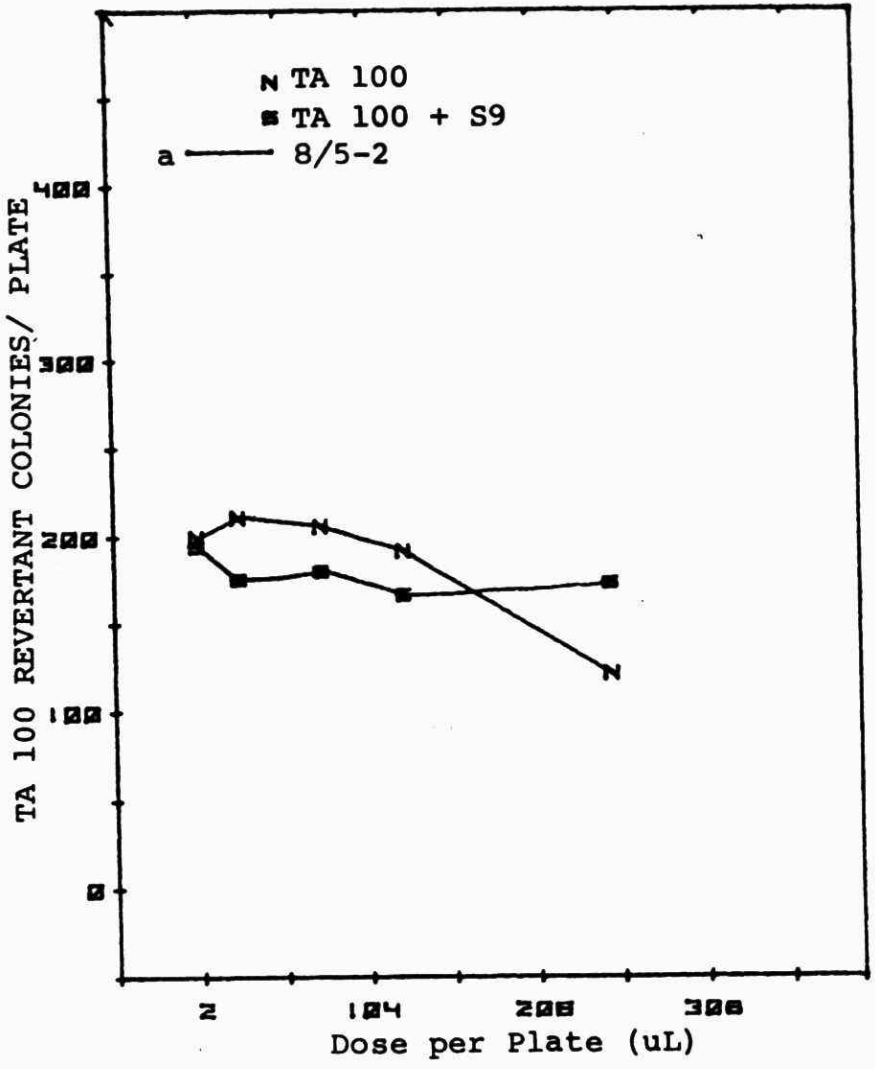
Appendix III K. Bacterial mutagenic response to the Steam Plant effluent concentrate of Dow Chemical of Canada Limited.
Positive controls: TA 98 + S9 and 2AF; a) 1270 @2ug, b) 1220 @2ug
TA 100 and MNNG; a) 369 @2ug, b) 1335 @2ug



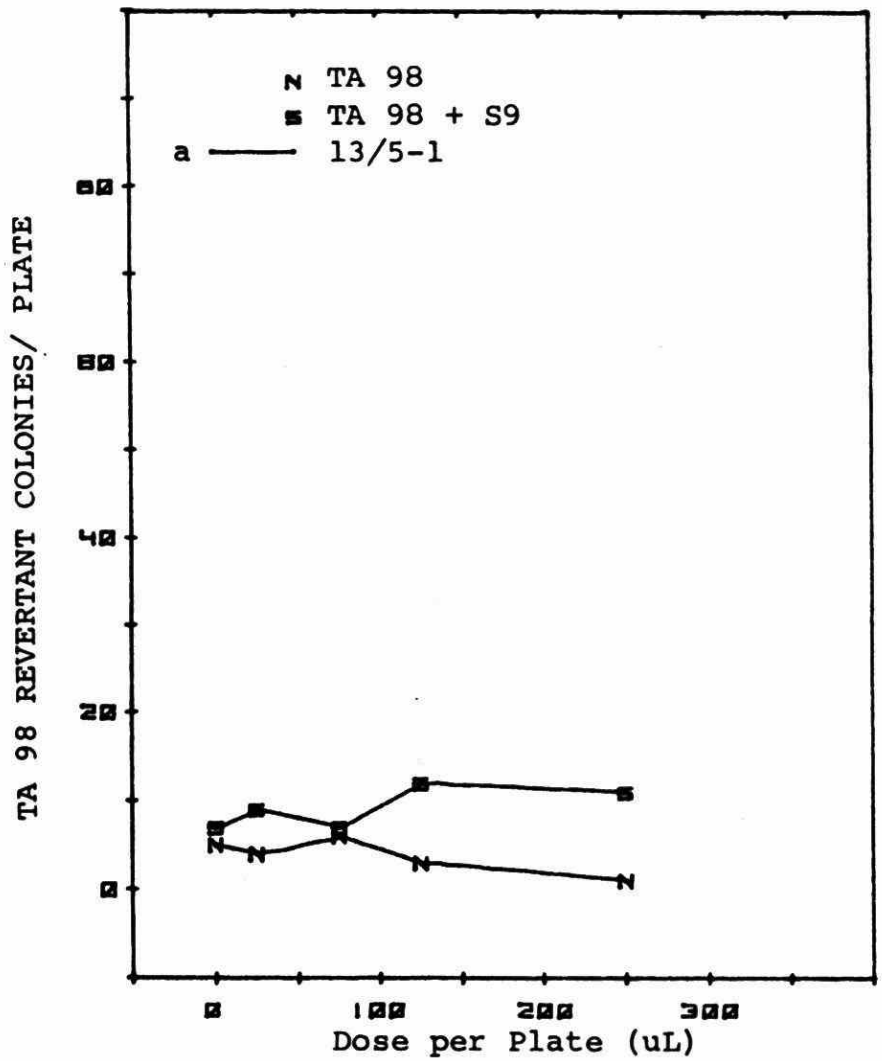


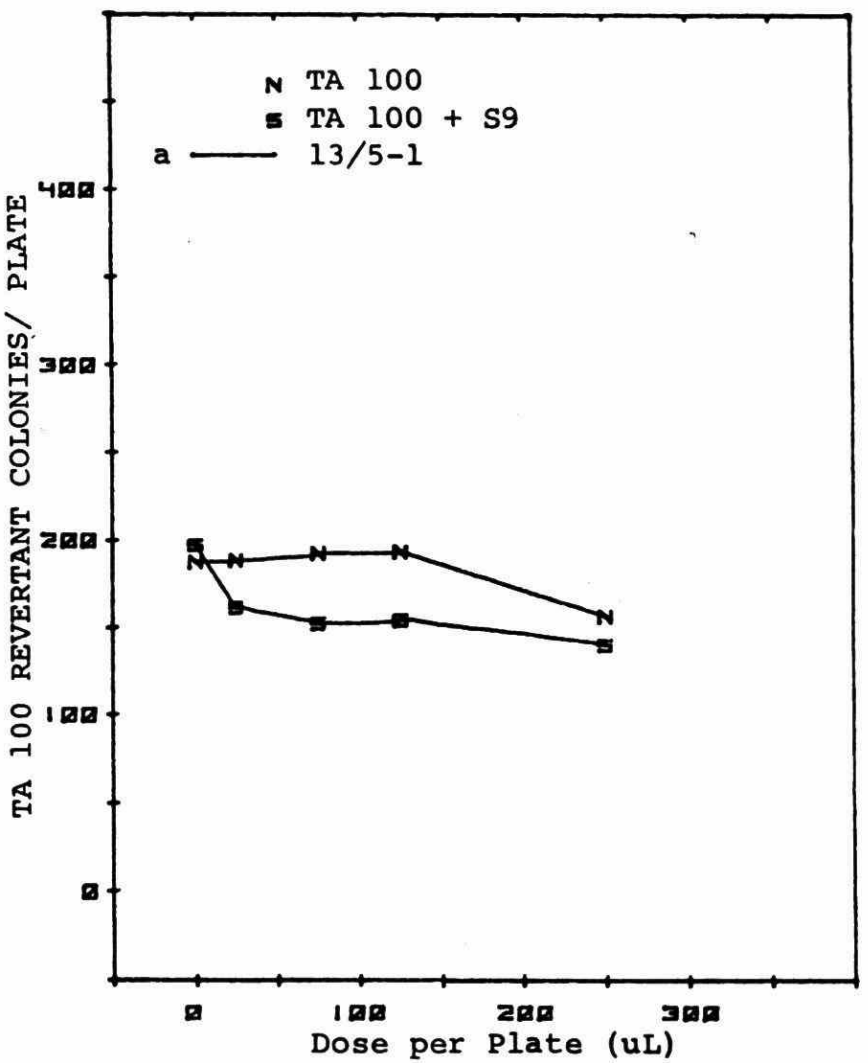
Appendix IV. Bacterial mutagenic response to the Final effluent concentrate of Sunoco Incorporated.
Positive controls: TA 98 + S9 and 2AF; a) 1165 @2ug, b) 380 @5ug
TA 100 and MNNG; a) 1196 @2ug, b) 1670 @5ug



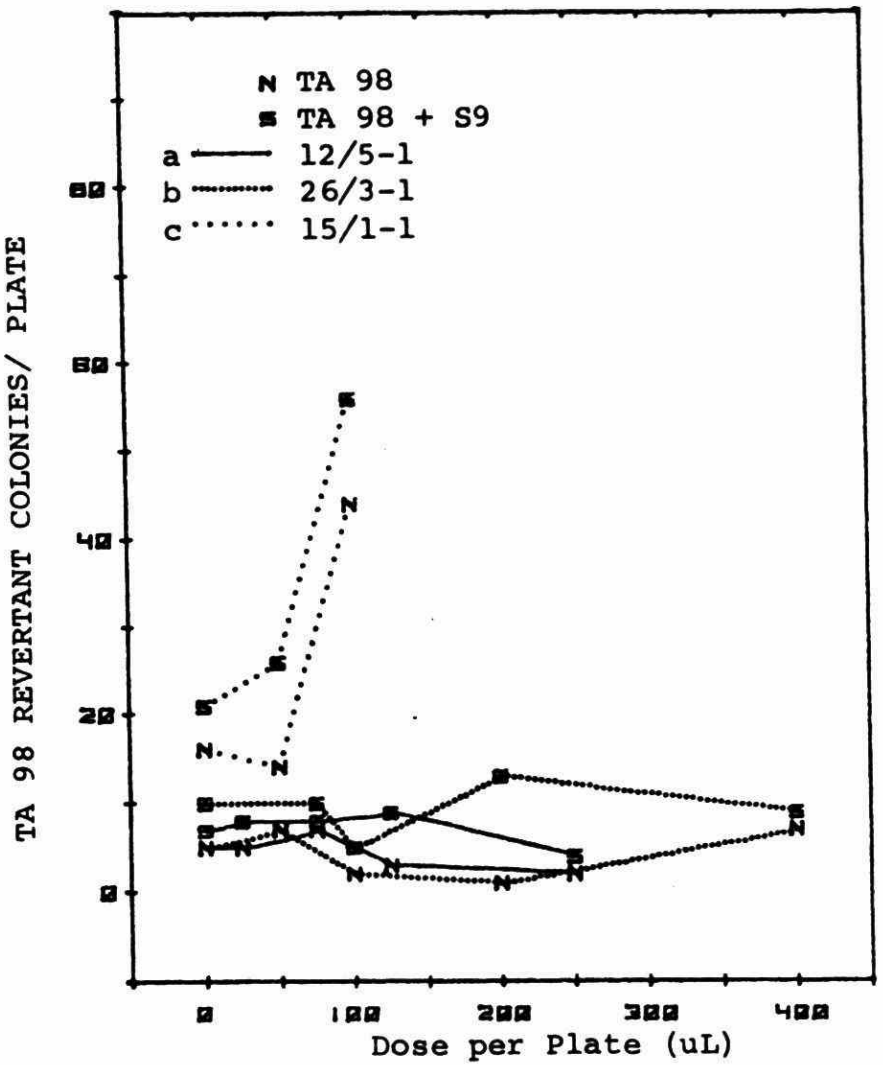


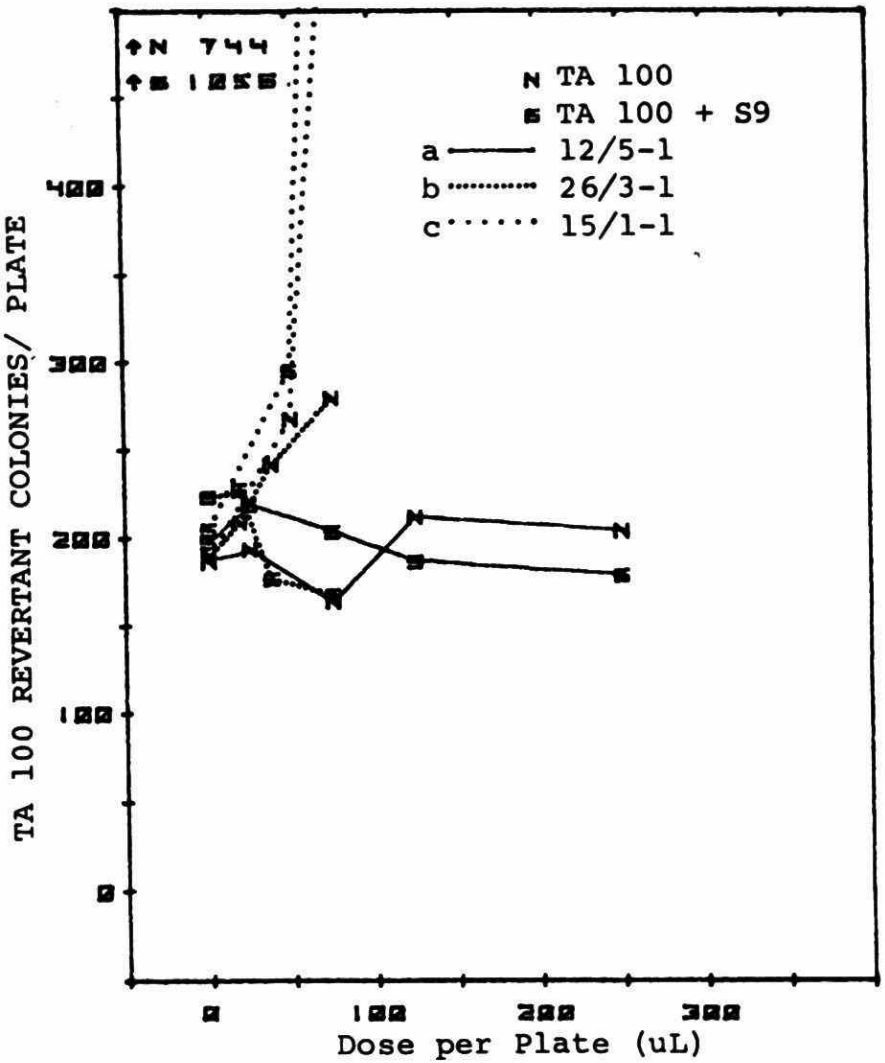
Appendix V A. Bacterial mutagenic response to the Final effluent concentrate of Shell Canada Limited.
Positive controls: TA 98 + S9 and 2AF; a) 1172 @2ug
TA 100 and MNNG; a) 2048 @2ug



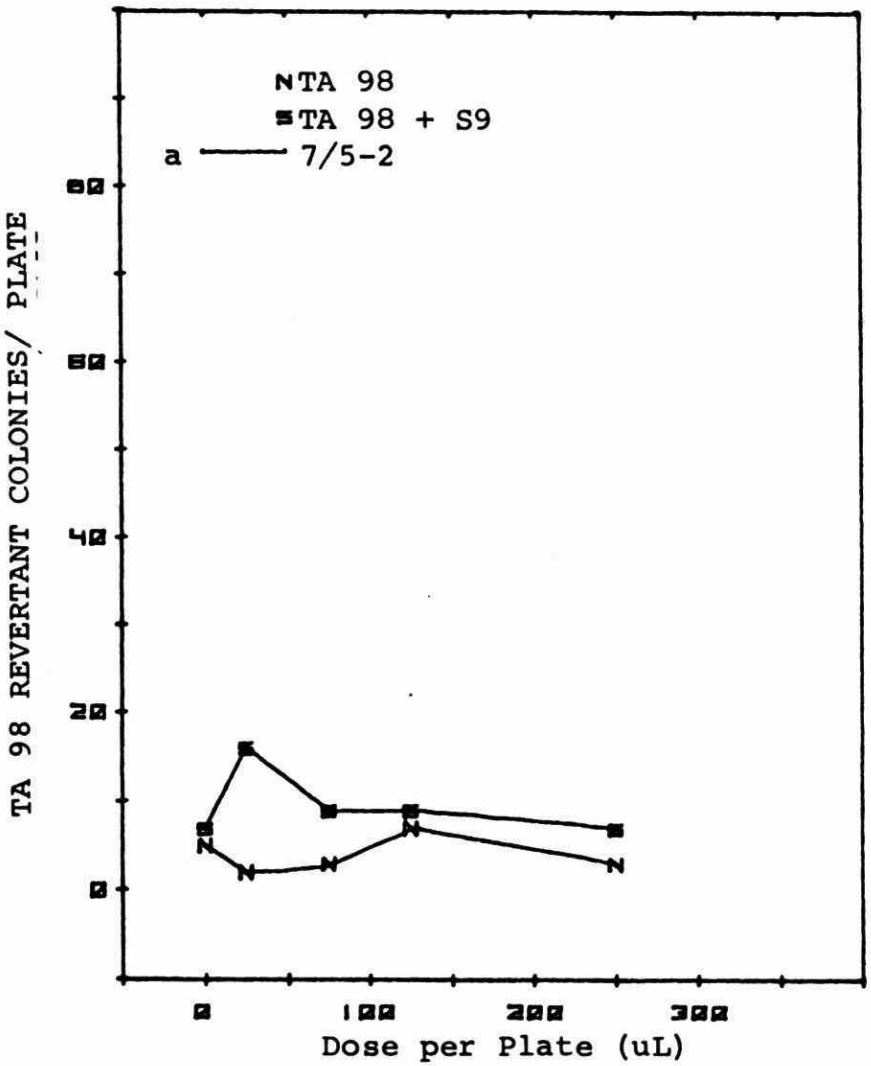


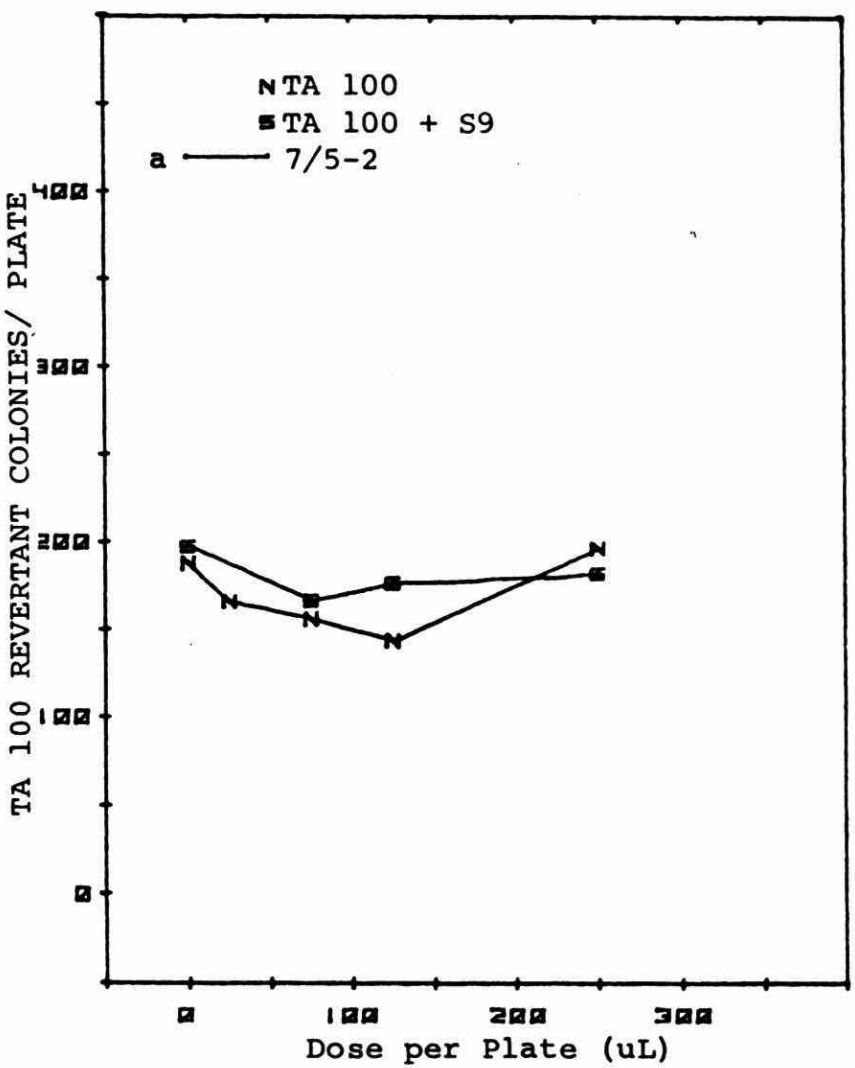
Appendix V B. Bacterial mutagenic response to the Contaminated Water effluent concentrate of Shell Canada Limited.
Positive controls: TA 98 + S9 and 2AF; a) 1363 @2ug
TA 100 and MNNG; a) 2683 @2ug



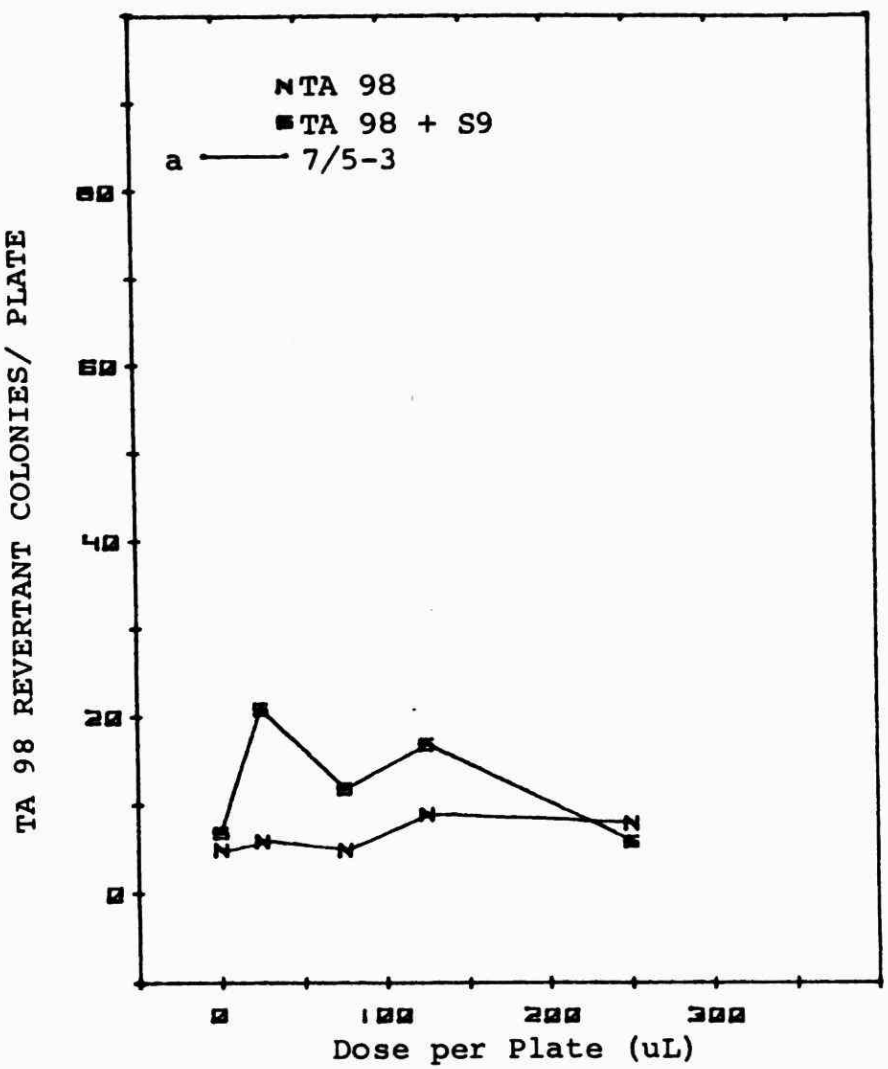


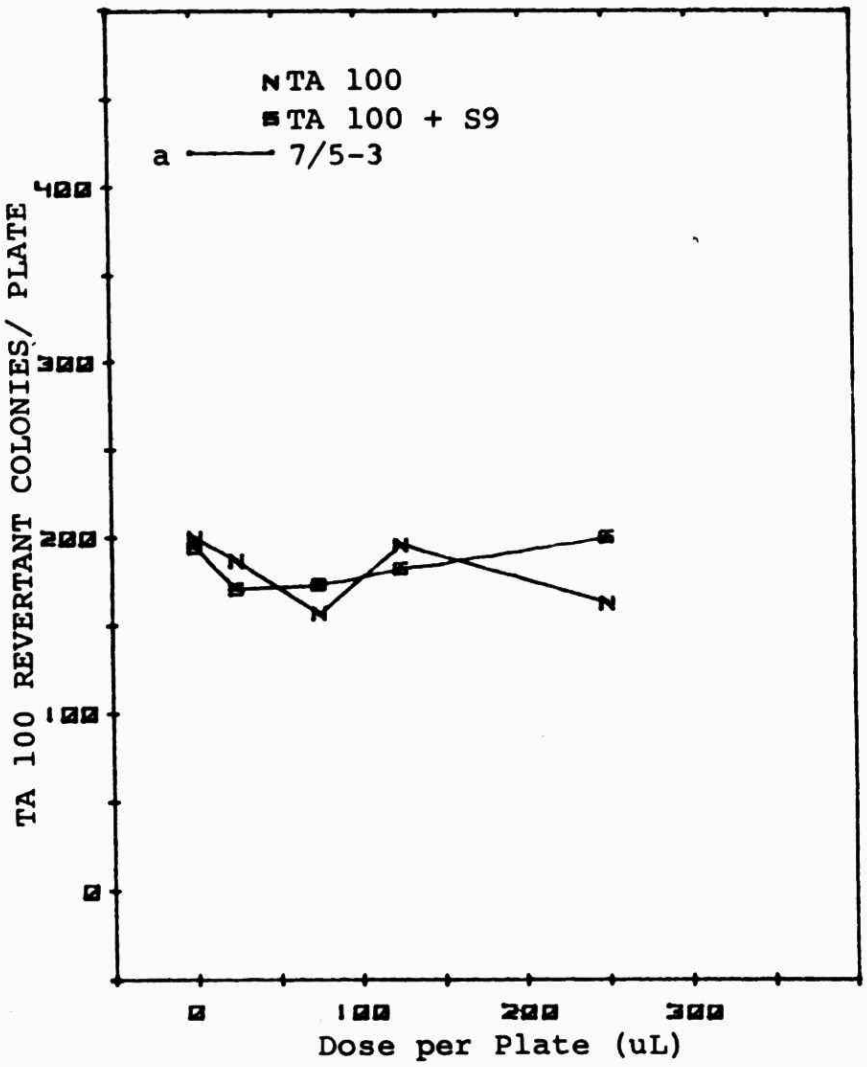
Appendix VI. Bacterial mutagenic response to the Final effluent concentrate of Ethyl Corporation of Canada Limited.
Positive controls: TA 98 + S9 and 2AF; a) 1363 @2ug, b) 2353 @4ug, c) 380 @5ug
TA 100 and MNNG; a) 2683 @2ug, b) 7503 @2ug, c) 1670 @5ug



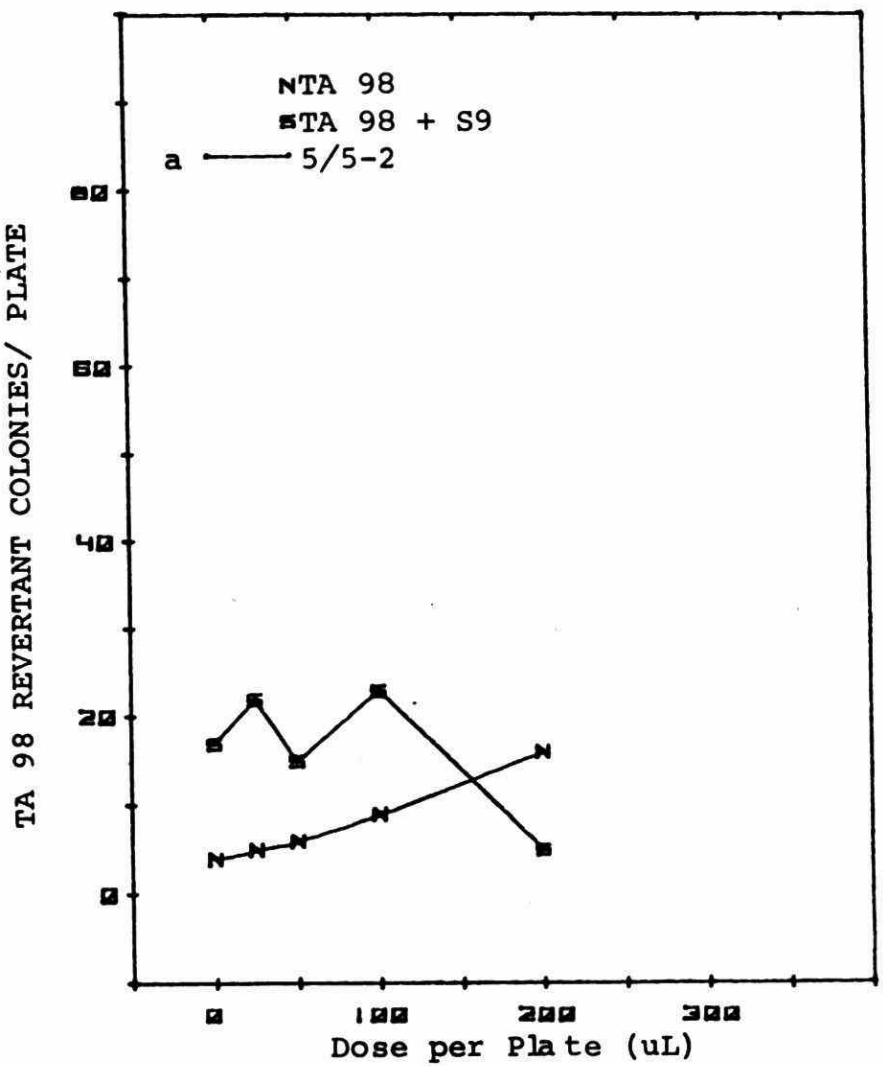


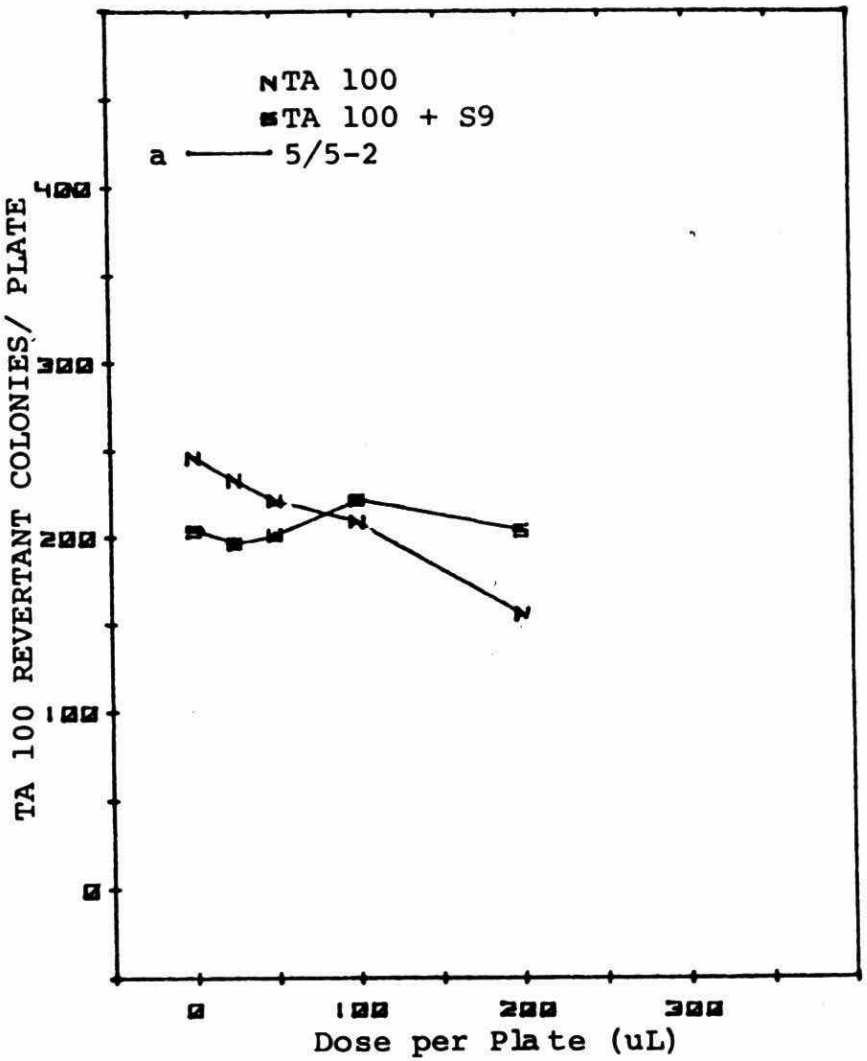
Appendix VII. Bacterial mutagenic response to the Final effluent concentrate of Dupont Canada Incorporated.
Positive controls: TA 98 + S9 and 2AF; a) 1363 @2ug
TA 100 and MNNG; a) 2683 @2ug





Appendix VIII. Bacterial mutagenic response to the Final effluent concentrate of Canadian Industries Limited.
Positive controls: TA 98 + S9 and 2AF; a) 1172 @2ug
TA 100 and MNNG; a) 2048 @2ug





Appendix IX. Bacterial mutagenic response to the Final effluent concentrate of Petrosar Limited.
Positive controls: TA 98 + S9 and 2AF; a) 1165 @2ug
TA 100 and MNNG; a) 1189 @2ug

APPENDIX X TO XIII

Appendices X through XIII contain the results of a screen of concentrates of industrial effluents for DNA damaging activity. The test used the tester strains Escherichia coli W3110 (containing DNA repair processes) and E. coli P3478 (lacking a DNA repair process). These two strains plus S-9 were also used in the screen. The samples was applied into a filter paper disk placed on the surface of an agar medium which was seeded with one of the tester strains. The results are expressed as the diameter (in mm) of the zone of inibition (ring of bacterial lawn clearing) minus the diameter of the disk.

In appendices X through XIII the following abbreviations were used:

C - negative control plates containing 100 µL dimethylsulphoxide in a 13 mm disk.

MNNG - N-methyl -N' - nitro -N - nitrosoguanidine. This mutagenic compound had DNA damaging activity and was preferentially more toxic to strain E. coli P3478. This compound was applied in a 7 mm disk.

CAMP - chloramphenicol (chloromycetin ™ 30 µL contained in a 7 mm disk. This compound theoretically was equally toxic to E. coli strains W3110 and P3478.

The concentrated effluent sample was applied in a 13 mm disk.

Appendix X. Escherichia coli DNA damaging activity in concentrated samples from Imperial Oil Enterprises Limited.

Effluent	Zone of Inhibition W3110 -S9	W3110 +S9	P3478 -S9	P3478 +S9
#3 Separator				
C	0	0	0	0
MNNG 10ug	5	5	18	18
CAMP 30ug	21	18	14	14
22/8-3,4 (100 uL)	0	0	0	0
Pressure Sewer				
C	0	0	0	0
MNNG 10ug	5	5	18	18
CAMP 30ug	21	18	14	14
29/8-5,6 (100 uL)	2.5	2.5	3.5	2.5
#9 Separator				
C	0	0	0	0
MNNG 10ug	6	6	18	17
CAMP 30ug	15	17	13	14
30/8-1,2 (100 uL)	0	0	0	0
Bio-oxidation System				
C	0	0	0	0
MNNG 10ug	5	5	18	18
CAMP 30ug	21	18	14	14
29/8-7,8 (100 uL)	0	0	0	0

Appendix XI. *Escherichia coli* DNA damaging activity in concentrated samples from Polysar Limited.

Effluent	Zone of Inhibition W3110 -S9	W3110 +S9	P3478 -S9	P3478 +S9
Township Ditch Effluent				
C	0	0	0	0
MNNG 5ug	3	4	18	18
CAMP 30ug	18	19	18	16
21/1-3 (140 uL)	0	0	0	0
54" Sewer				
C	0	0	0	0
MNNG 5ug	4	4	13	12
CAMP 30ug	17	17	17	17
18/1-2 (100 uL)	0	0	0	0
C	0	0	0	0
MNNG 10ug	5	6	20	22
CAMP 30ug	18	17	17	15
23/8-1,2 (100 uL)	0	0	0	0
66" Sewer				
C	0	0	0	0
MNNG 10ug	6	6	18	17
CAMP 30ug	17	17	15	13
31/8-3,4 (100 uL)	0	0	0	0
Stereo API				
C	0	0	0	0
MNNG 10ug	7	6	18	18
CAMP 30ug	20	19	16	15
22/8-1,2 (100 uL)	2	2	3	5

Appendix XI. continued.

Effluent	Zone of Inhibition W3110 -S9	W3110 +S9	P3478 -S9	P3478 +S9
72" Sewer				
C	0	0	0	0
MNNG 10ug	6	6	18	17
CAMP 30ug	17	17	15	13
31/8-5,6 (100 uL)	0	0	0	0
Influent				
Township Ditch Influent				
C	0	0	0	0
MNNG 10ug	3	4	18	18
CAMP 30ug	18	19	18	16
21/1-2 (140 uL)	0	0	0	0

Appendix XII. *Escherichia coli* DNA damaging activity in concentrated samples from Dow Chemical of Canada Limited.

Effluent	Zone of Inhibition W3110 -S9	W3110 +S9	P3478 -S9	P3478 +S9
42" Sewer				
C	0	0	0	0
MNNG 10ug	4	4	20	21
CAMP 30ug	19	17	17	15
28/8-7,8 (100 uL)	0	0	0	0
48" Sewer				
C	0	0	0	0
MNNG 10ug	4	4	20	21
CAMP 30ug	19	17	17	15
27/8-5,6 (100 uL)	0	0	0	0
Acid Tile				
C	0	0	0	0
MNNG 10ug	4	4	20	21
CAMP 30ug	18	17	16	16
24/8-1,2 (100 uL)	3	0	5	3
54" Sluice				
C	0	0	0	0
MNNG 10ug	11	11	21	22
CAMP 30ug	25	25	19	18
29/8-1,2 (100 uL)	0	0	0	0
2nd Street Sewer				
C	0	0	0	0
MNNG 10ug	4	4	20	21
CAMP 30ug	19	17	17	15
27/8-1,2 (100 uL)	0	0	3	4

Appendix XII. continued.

Effluent	Zone of Inhibition W3110 -S9	W3110 +S9	P3478 -S9	P3478 +S9
3rd Street Sewer				
C	0	0	0	0
MNNG 10ug	11	11	21	22
CAMP 30ug	25	25	19	18
28/8-11,12 (100 uL)	0	0	0	0
Direct Oxidation of Ethylene Oxide (DOEO)				
C	0	0	0	0
MNNG 10ug	11	11	21	22
CAMP 30ug	25	25	19	18
28/8-9,10 (100 uL)	0	0	0	0
4th Street Sewer				
C	0	0	0	0
MNNG 10ug	7	6	18	18
CAMP 30ug	20	19	16	15
27/8-3,4 (100 uL)	1	2	0	0

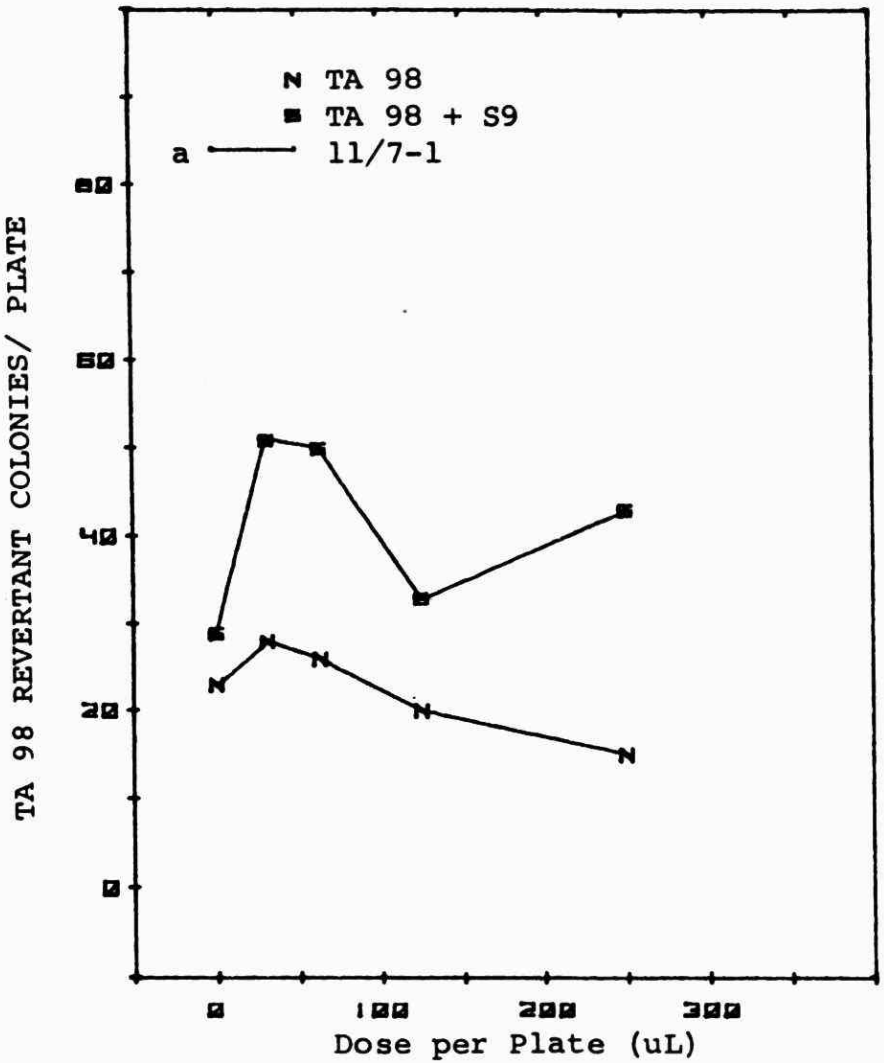
Appendix XIII. *Escherichia coli* DNA damaging activity in concentrated samples of industrial effluents

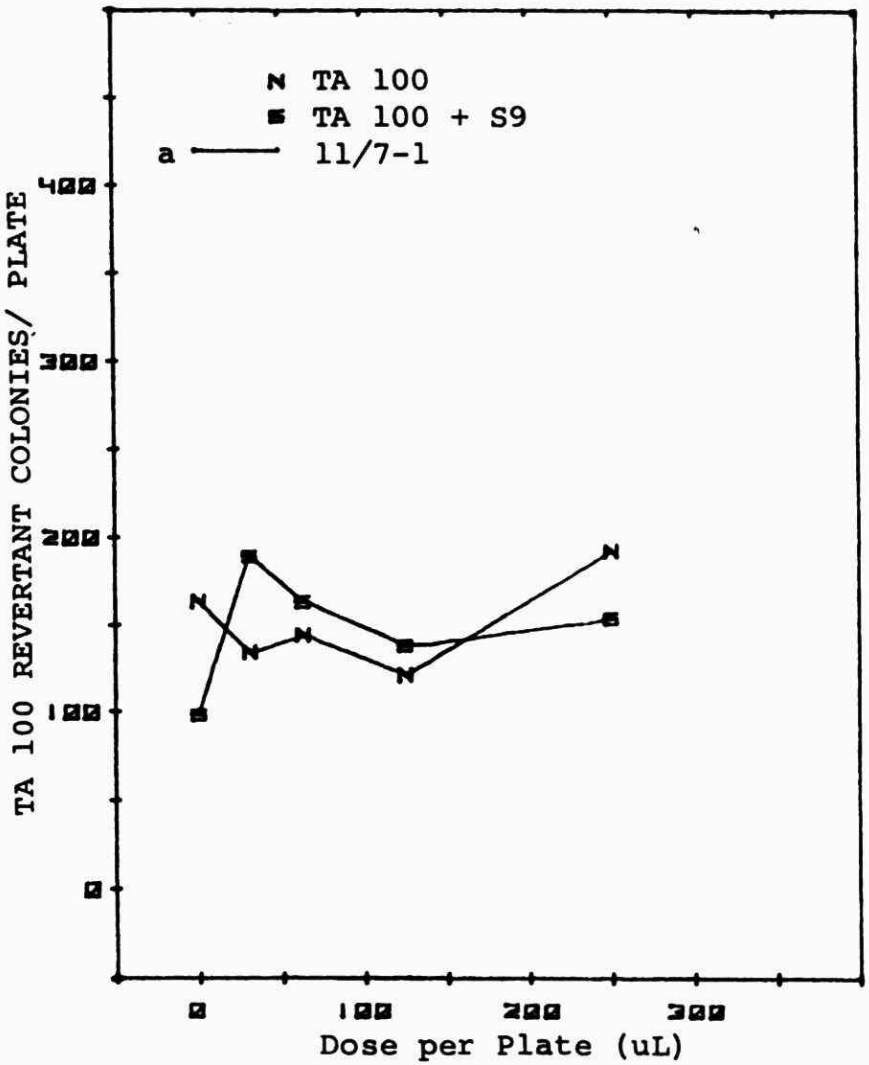
Industry	Zone of Inhibition W3110 -S9	W3110 +S9	P3478 -S9	P3478 +S9
Sunoco Incorporated Final Effluent				
C	0	0	0	0
MNNG 5ug	27*	27	38	38
CAMP 30ug	17	18	16	15
9/1-1 (140 uL)	0	0	0	0
Ethyl Corporation of Canada Limited Final effluent				
C	0	0	0	0
MNNG 5ug	3	2	12	14
CAMP 30ug	19	18	16	16
15/1-1 (100 uL)	0	0	0	0
C	0	0	0	0
MNNG 5ug	5	5	14	13
CAMP 30ug	17	18	18	19
29/1-5 (100 uL)	0	0	0	0
Dupont Canada Incorporated Final Effluent				
C	0	0	0	0
MNNG 5ug	27*	27	38	38
CAMP 30ug	17	18	16	15
10/1-1 (100 uL)	0	0	0	0
C	0		0	0
MNNG 5ug	4		12	12
CAMP 30ug	19		17	14
24/1-3 (100 uL)	0		0	0

* MNNG applied directly to plate.

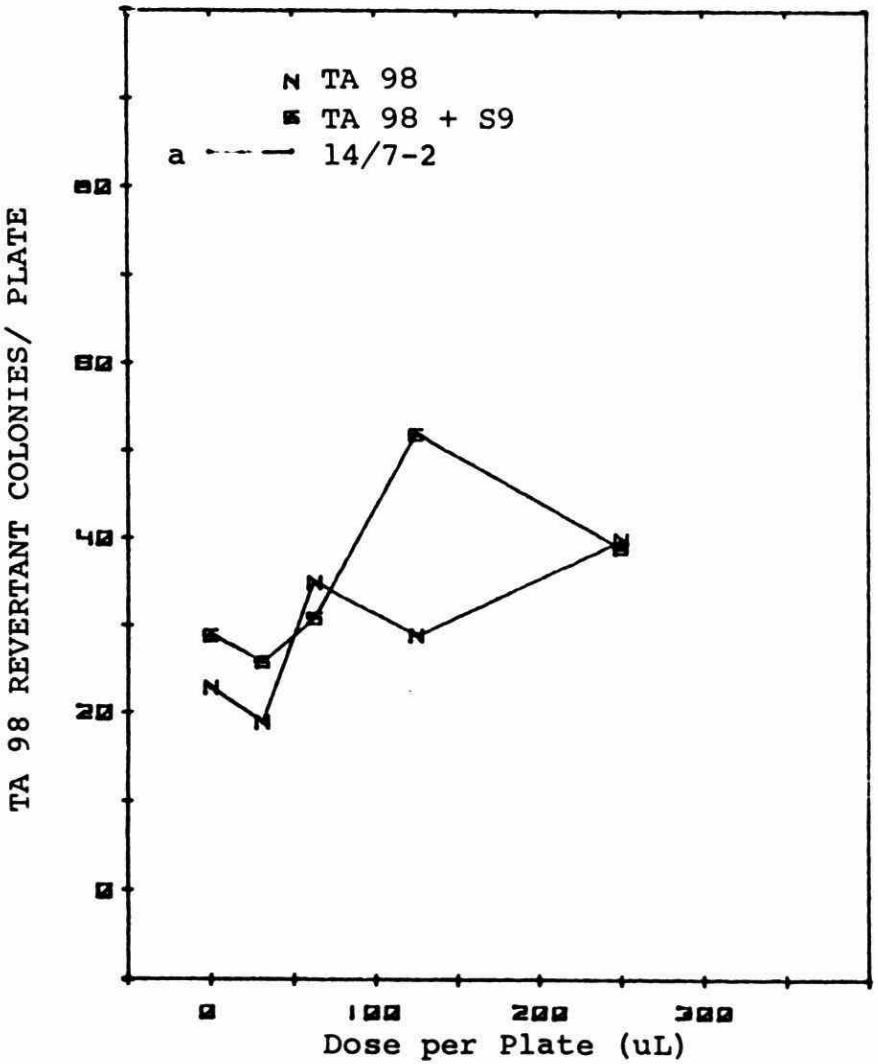
Appendix XIII. continued.

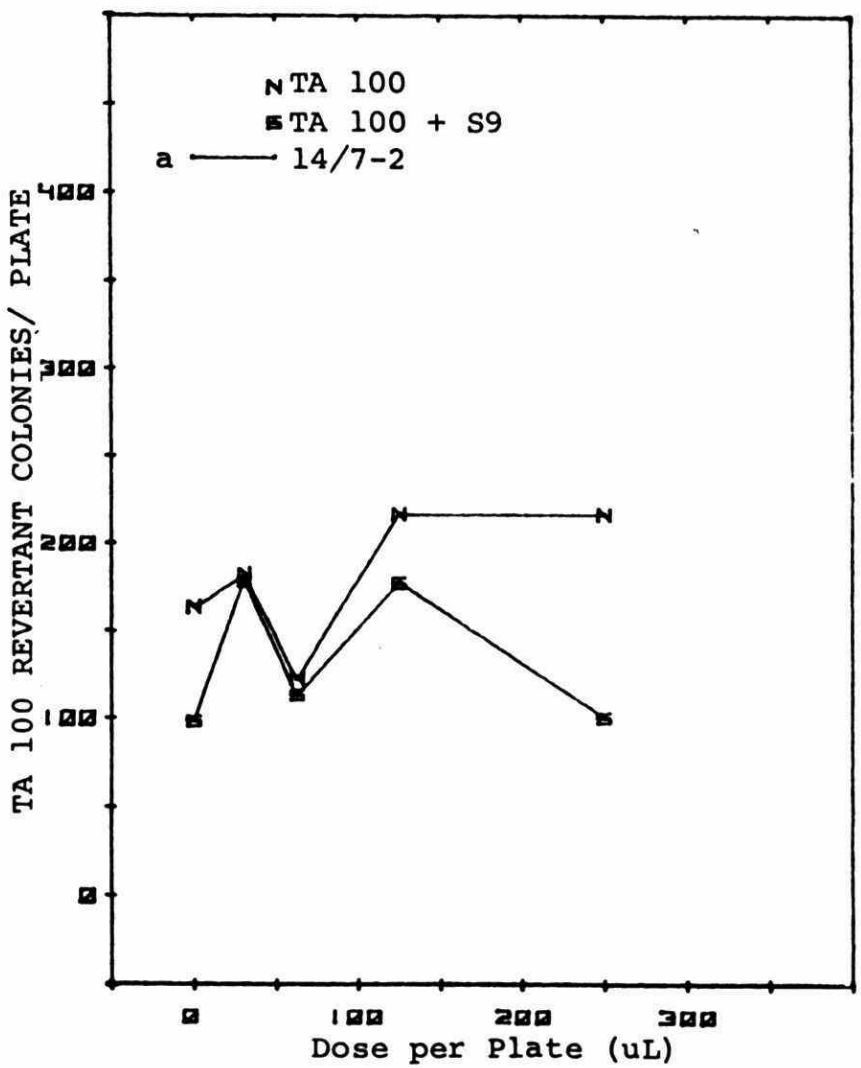
Industry	Zone of inhibition W3110 -S9	W3110 +S9	P3478 -S9	P3478 +S9
Canadian Industries Limited Final Effluent				
C	0	0	0	0
MNNG 5ug	3	4	18	18
CAMP 30ug	18	19	18	16
21/2-3 (140 uL)	0	0	0	0



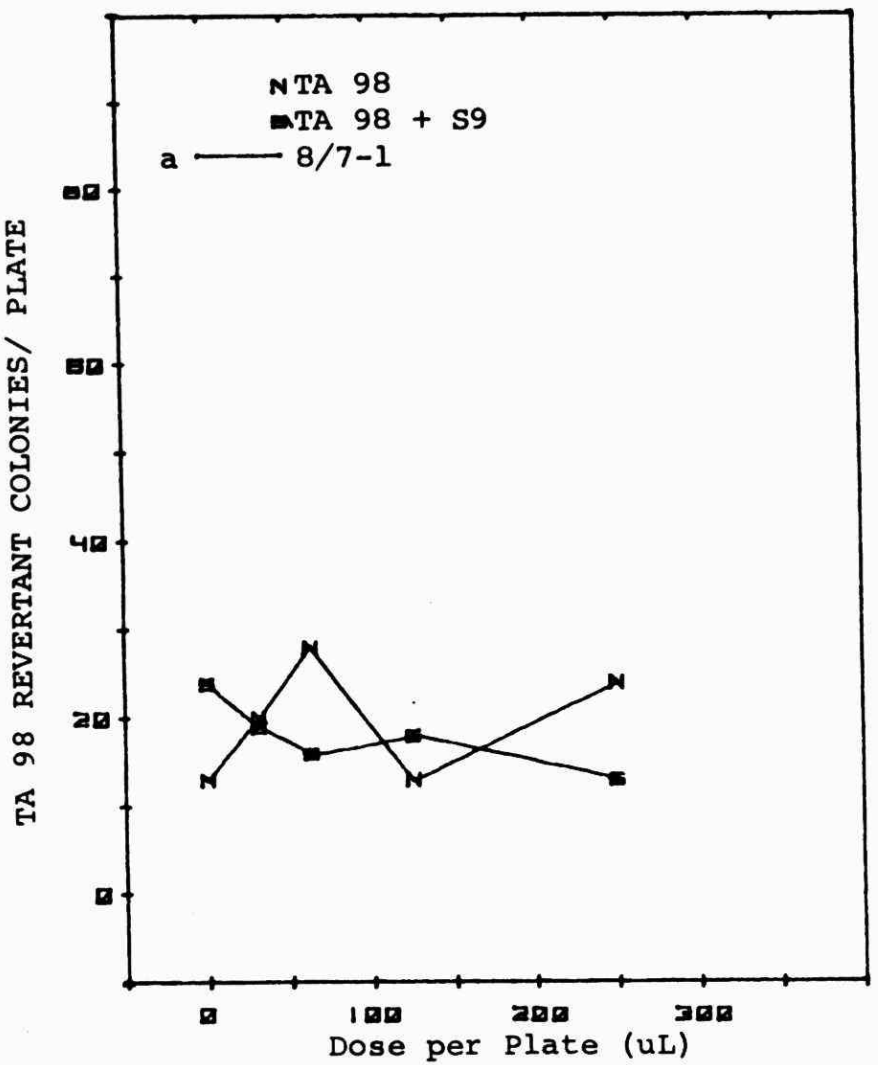


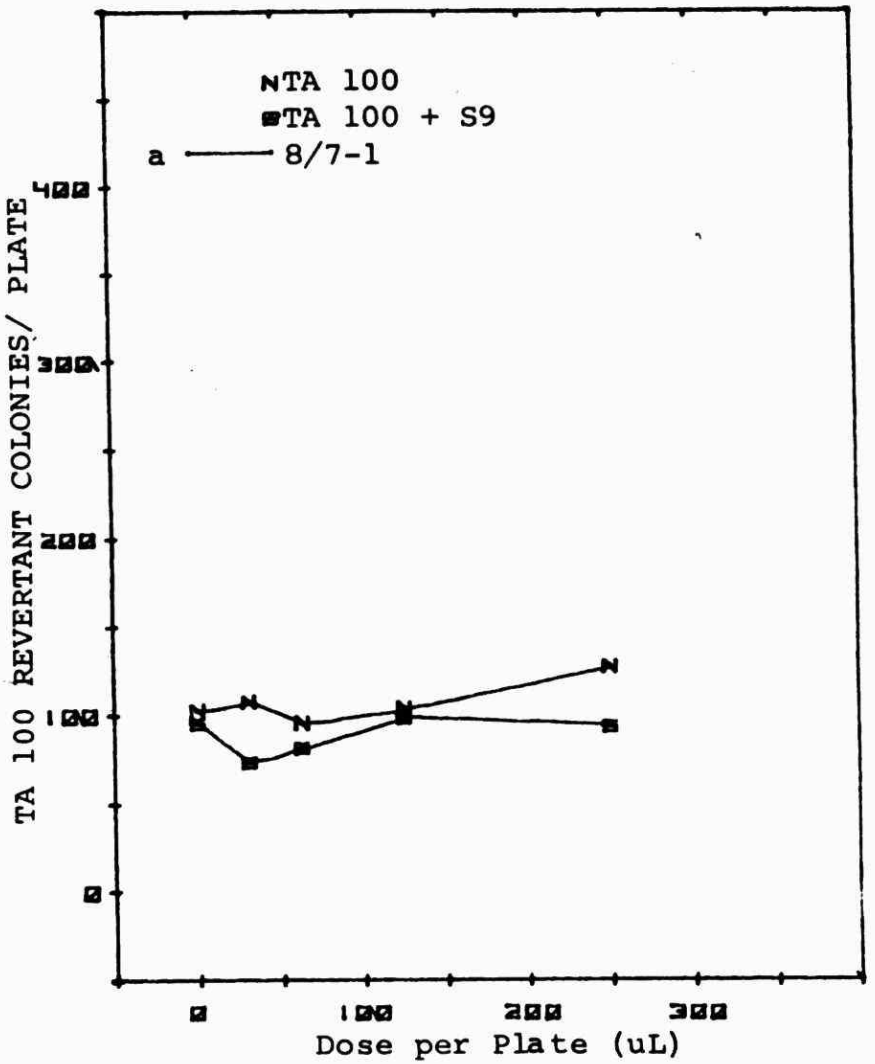
Appendix XIV A. Bacterial mutagenic response to the St. Clair River sample concentrate from upstream of Polysar Limited.
Positive controls: TA 98 + S9 and 2AF; a) 274 @2ug
TA 100 and MNNG; a) 1325 @2ug



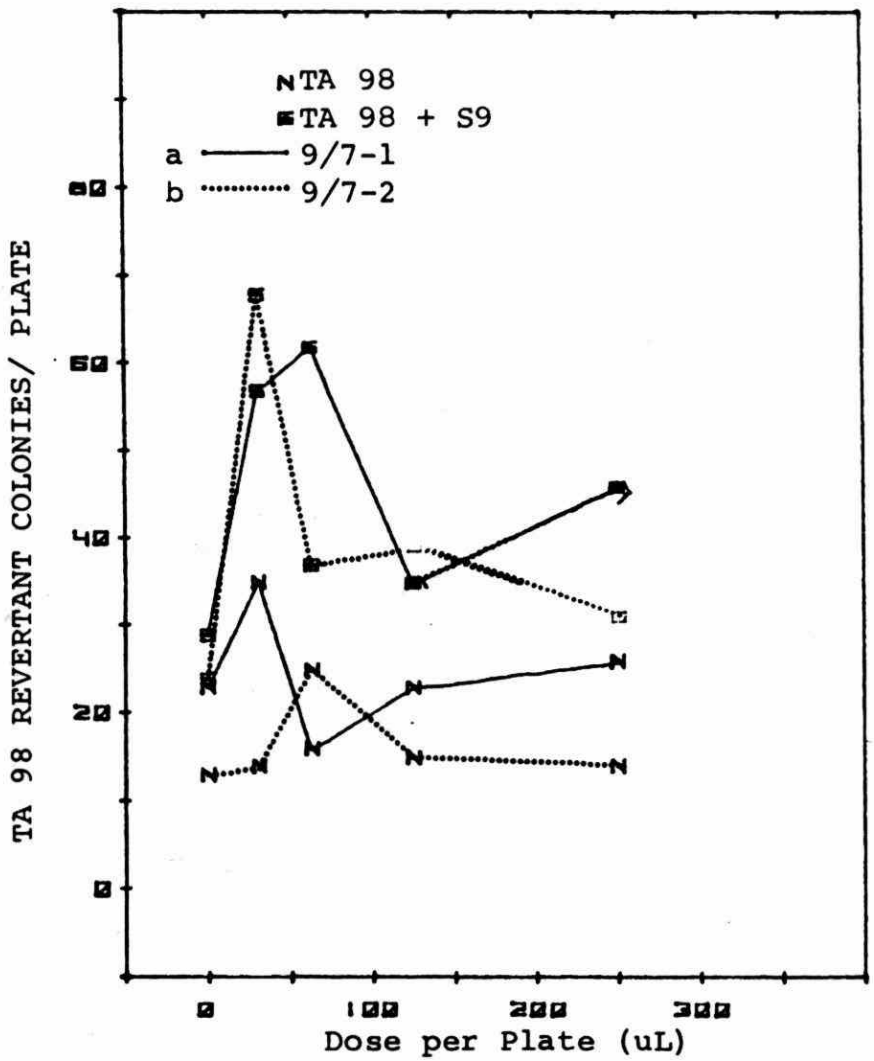


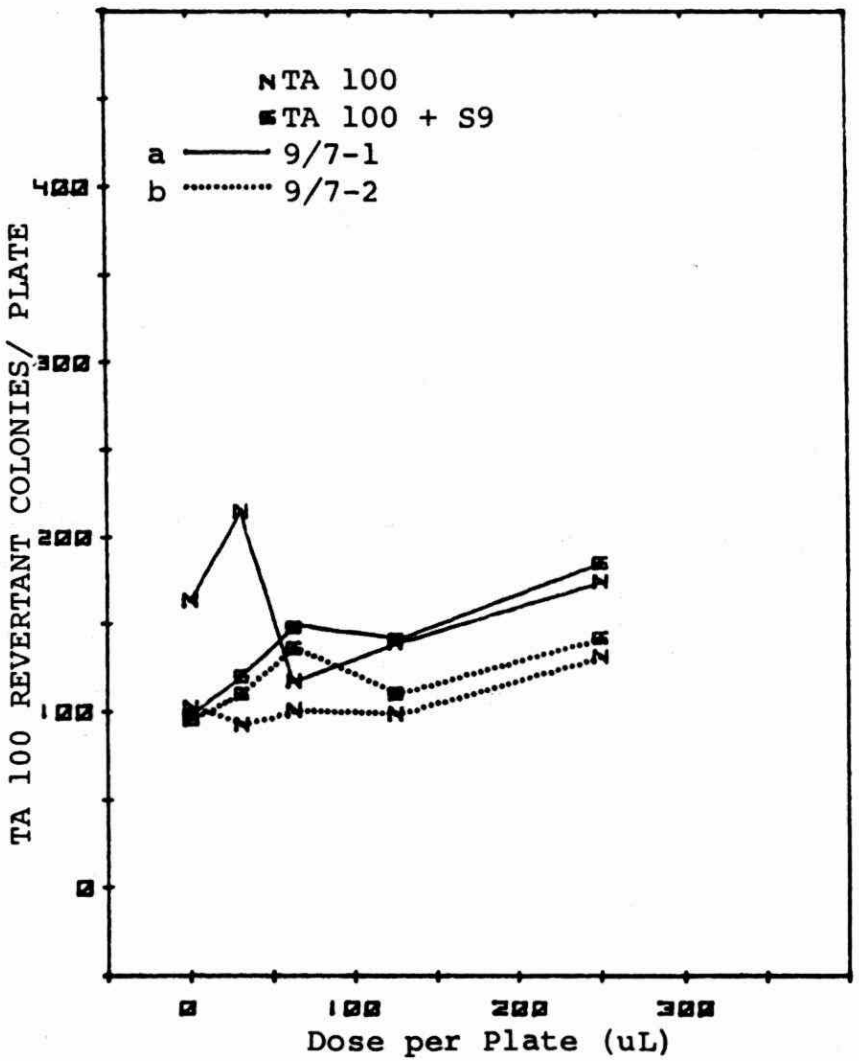
Appendix XIV B. Bacterial mutagenic response to the St. Clair River sample concentrate from the Plume, Polysar Limited.
Positive controls: TA 98 + S9 and 2AF; a) 274 @2ug
TA 100 and MNNG; a) 1325 @2ug



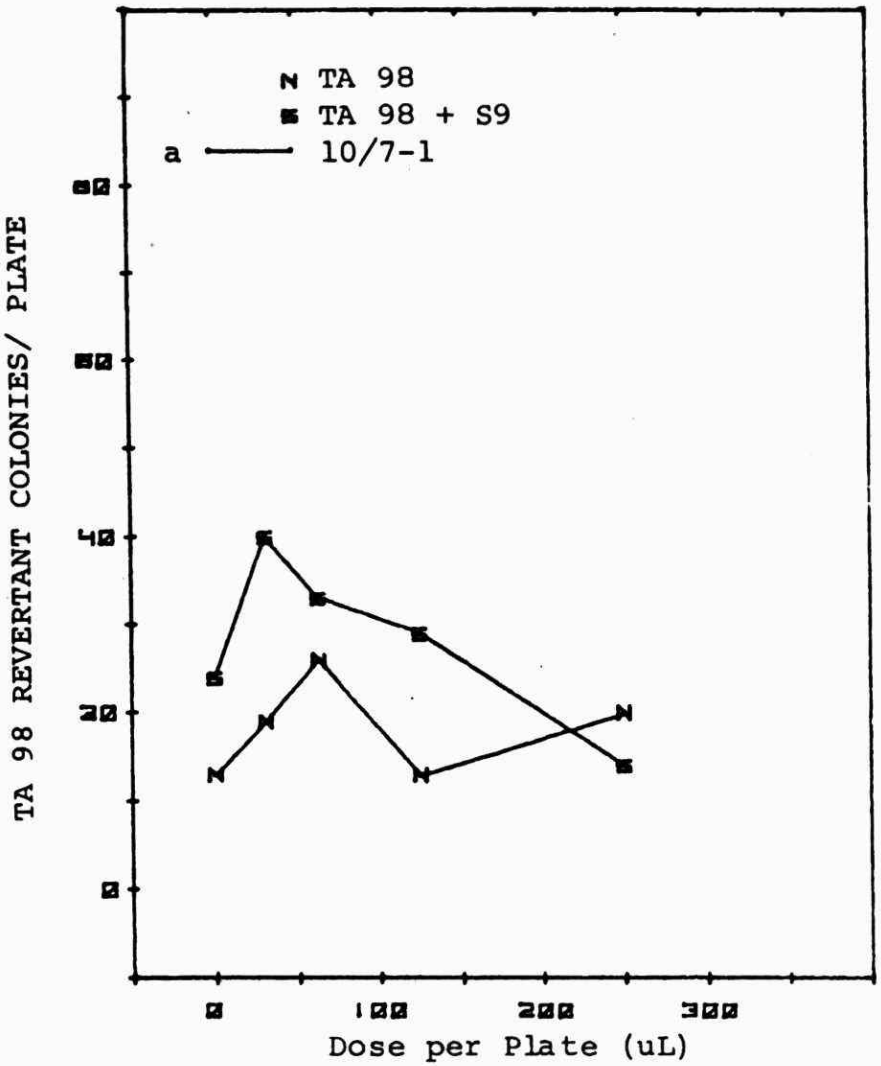


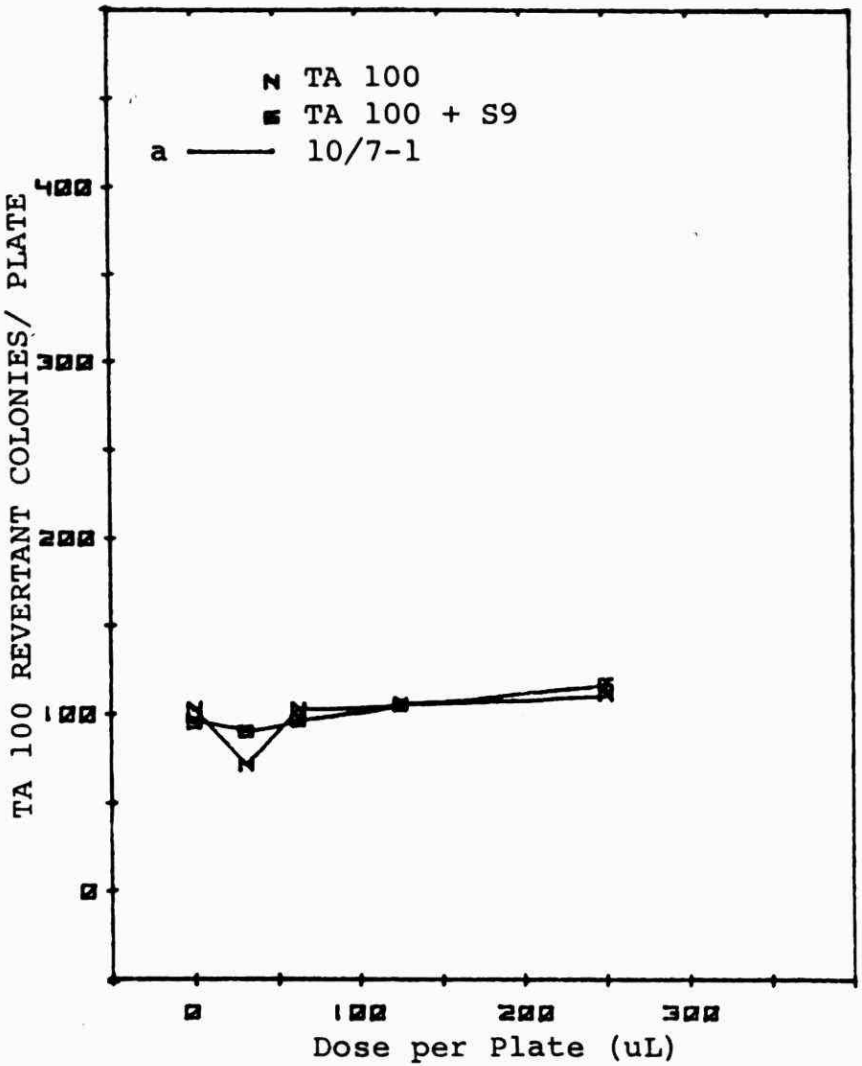
Appendix XIV C. Bacterial mutagenic response to the St. Clair River sample concentrate from the Plume, Dow 1st Street Complex.
Positive controls: TA 98 + S9 and 2AF; a) 1203 @2ug
TA 100 and MNNG; a) 451 @2ug





Appendix XIV D. Bacterial mutagenic response to the St. Clair River sample concentrate from the Plume, Dow 2nd Street Sewer.
Positive controls: TA 98 + S9 and 2AF; a) 341 @2ug, b) 1203 @2ug
TA 100 and MNNG; a) 1325 @2ug, b) 451 @2ug





Appendix XIV E. Bacterial mutagenic response to the St. Clair River sample concentrate from downstream of the Dow 4th Street Sewer.
Positive controls: TA 98 + S9 and 2AF; a) 1203 @2ug
TA 100 and MNNG; a) 451 @2ug